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HERPETOMONAS MUSCAE-DOMESTICAE, ITS BEHAVIOR AND EFFECT IN LABORATORY ANIMALS

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While making some observations on the habits of blood sucking flies, at a large dairy during the summer of 1921, the writer noticed that many of the house flies frequenting cattle were engorged with blood. A microscopc examination of the alimentary tract proved that the blood was obtained from cows. Closer observations revealed the fact that house flies frequently feed at the punctures recently deserted by *Stomoxys calcitrans* or *Haematobia serrata*. It was noticed that house flies hovered near stable or horn flies when feeding and immediately pounced upon the wounds inflicted by these insects as soon as the biting flies had flown away to rest and digest their meals. The house flies in turn sucked blood until engorged. These observations demonstrated certain possibilities under which house flies might act as transmitters of microörganisms and parasites found at times within the peripheral circulation.

Darling in 1912 proved that *Musca domestica* was able to transmit *Trypanosoma hippicum* to healthy mules after feeding on the sores of diseased ones.

Patton and Cragg in 1913 showed that some non-biting members of the genus Musca, like *M. pattoni,* associated themselves around cattle with such biting forms as Haematopota, Stomoxys, Philaematomyia, and other forms. Most of the non-biting species feed only on blood and depend entirely on the biting species for the preparation of their food. Patton and Cragg further noticed that the non-biters were not house flies, but species which are seldom found away from cattle. In the Philippines in 1912, Mitzmain observed a close association between non-biting flies and Stomoxys, but the non-biting species were not identified.

Since no protozoa were at this time found in biting flies, and since the house fly offered possibilities for the dissemination of blood parasites, it became important to make a careful study of the protozoan

fauna found in the alimentary tract of such flies; to attempt to cultivate these protozoa, and determine their pathogenic properties. Three species of flagellates were found in the digestive tract of house flies during the summer of 1921, but only one of these will receive consideration at this time.

Herpetomonas muscae-domesticae Burnett was by far the most prevalent flagellate inhabiting the digestive tract of adult house flies. When the Herpetomonad was present at all, it was nearly always there in enormous numbers; sometimes the entire intestinal contents seemed to consist of little else. In order to avoid falling into an error, the writer considered only those flies parasitised which showed the true flagellated form. Possible preflagellate or postflagellate types of the parasite were not tabulated for the reason that some of these are easily confused with certain stages of the two other species obtained and since cultivated by me in pure culture.

Dissections of house flies from June 1 until the middle of November gave the following percentages of infected individuals.

	Number dissected	Infection
June	80	12.5 per cent.
July	110	41.0 per cent.
August	125	52.0 per cent.
September	95	21.0 per cent.
October	50	4.0 per cent.
November	50	0 per cent.

Judging from the writings of others, the percentages of infected flies found here in New Jersey correspond more nearly to the percentages obtained by workers in warmer climates. In Madras, Patton found 100 per cent. infected. In Syria, Wenyon states that the majority of the house flies harbored *H. muscae-domesticae*. Prowazek found 8 per cent. infection at Rovigno, while Franchini and Mantovani found a 3 per cent. infection near Boulogne. Dunkerly and Hewitt were unable to find the parasite in England and the writer knows of no records in America.

Franchini and Mantovani (1915) discovered their infected flies on farms in the environment of Boulogne. Flies caught in the houses of Boulogne showed no Herpetomonads. This observation was independently verified by the writer during the summer. In going over his notes he does not find a single record of a dissection of a fly caught within dwelling houses that revealed any flagellates whatever. All of the parasitised flies were caught in cow barns and stables. This seems to suggest a rather close association between the Herpetomonad and domestic animals.

The flagellate under discussion received much attention by other workers on account of its similarity to the parasite of Kala Azar. Prowazek (1904), Patton (1908-1909), Porter (1909), MacKinnon (1910), Wenyon (1911 and 1913) fully described the life history and cytology of the flagellate from films and sections of the intestines of house flies. During all of this time much discussion arose concerning various morphological details. Prowazek, Lingard and Jennings, claimed that the Herpetomonad had a double flagellum, while Léger, Patton, Porter, MacKinnon, and Wenyon insisted that the animal had a single flagellum and that a double flagellum represents the beginning of longitudinal division of the parasite.

The present writer obtained some excellent films from flies fixed and stained with Giemsa which seem to prove that the flagellum is single and that the so-called "double nature" of the flagellum is a division phenomenon. Figures 1, 2, 3 and 4, show various types of the mature and maturing Herpetomonad. The trophonucleus is situated near the center of the lancet shaped body. The blepharoplast is located at the anterior flagellar end. Figure 5 represents the flagellum in the act of splitting longitudinally accompanied or preceded by the division of the blepharoplast. Figure 6 shows a further advance consisting in the division of the trophonucleus accompanied or succeeded by cell division. A separation of the two halves of the flagellum is noticed at the anterior end of the protozoan. Figure 7 is the last stage preceding the separation of the two daughter halves. Figure 3 shows a deeply staining thread which runs backward from the kinetonucleus. That this thread is spirally coiled as claimed by Prowazek could not be determined. In the writer's specimens the thread appears nearly straight. Some individuals were found which showed a distinct vacuole at the extreme anterior end (Fig. 3). Figures 8 and 9 represent other morphological forms of the same species of Herpetomonad frequently encountered. A cytopharynx as found by Wenyon (1913) was not observed. Moreover, whole or even parts of bacteria are not found within the endoplasm. It is highly probable that the protozoan is nourished through osmotic diffusion currents.

Since the morphology and life history of the flagellate has been completely worked out by others, the writer attempted very little in this direction. In anticipation of experimental work, however, it was thought important to identify the species here in New Jersey with the European form. Notwithstanding various difficulties encountered in interpreting conflicting statements in the literature concerning the morphology of *Herpetomonas muscae-domesticae* (often also referred to as *Leptomonas muscae-domesticae* or *Crithidia muscae-domesticae*), the writer is fairly certain that he is dealing if not with the identical species, at least with a variety very closely related to the form or forms

studied by Prowazek, Patton, Wenyon, Franchini, Mantovani, and other European and Asiatic workers.

In 1915, a fresh impetus was imparted to the whole Herpetomonad work by Laveran and Franchini. These investigators found a Herpetomonad in the mouse flea resembling *H. muscae-domesticae* in many respects. They were able to cultivate the flagellate in pure culture on the Nicolle, Novy and McNeal medium. Laveran and Franchini found Leishmaniform bodies in their cultures after a few days which later developed into true Herpetomonads. Later, in 1919, these same observers produced a typical form of Leishmaniasis in white mice by inoculating them with cultures of the Herpetomonad found in the intestine of the mouse flea. Rats also proved susceptible and developed Leishmaniasis, better called Herpetomaniasis as suggested by Fantham and Porter (1916). The disease was also reproduced by feeding the parasites. The Herpetomonads of *Anopheles maculipennis* and *Melophagus ovinus* produced similar conditions. In 1919, Chatton also cultivated a Herpeomonad from the dog flea and reproduced Leishmaniasis in dogs.

In all of these experimental cases, the animals were inoculated or fed with the flagellated forms found in culture. After periods varying between six weeks and two and a half months, the animals began to show symptoms such as fever, emaciation, with loss of appetite and weight. Often Leishmaniform and sometimes flagellated forms were found in the blood. Autopsies of very sick animals, and those that died of the disease usually showed an enlargement of the spleen in which the rounded or oval Leishmaniform bodies were found. These bodies also proved quite numerous in the bone marrow. The liver rarely revealed any parasites and the other organs never.

In 1916, Fantham and Porter reported a large number of experiments with Herpetomonads and Crithidia obtained from the digestive tract of a variety of insects. With these flagellates they were able to produce Herpetomoniasis in mice, dogs, canaries, sparrows, martins, grass snakes, lizards, frogs, toads, and sticklebacks. From the experiments the authors concluded that:

"Herpetomoniasis or Leishmaniases can be induced in various warm and cold blooded vertebrates when the latter are inoculated or fed with Herpetomonads occurring in the digestive tracts of various insects. The infection produced and the protozoan parasites found in the vertebrates resemble those of human and canine Leishmaniases." "The disease induced may run an acute or a chronic course. In the acute cases among the vertebrates, the flagellate form of the parasite was the more obvious at death. In chronic cases, non-flagellate forms of the parasite were more numerous."

Fantham and Porter argue that since the flagellated stages of *Leishmania donovani* and *L. tropica* are now known, the links com-

pleting the evidence that a Leishmania is a Herpetomonas are complete. They further express the belief "that Leishmaniases are invertebrate-borne Herpetomoniases, and that these maladies have been evolved from flagellates of invertebrates (especially Herpetomonads of insects) which have been able to adapt themselves to life in vertebrates."

In 1915 Franchini and Mantovani stated that they were able to produce Leishmaniasis or Herpetomoniasis in rats with *H. muscae-domesticae,* the house fly parasite. Besides reproducing the usual symptoms of Herpetomoniasis and the Leishmaniform stages in the involved organs, they were further able to cultivate the organism indirectly. House flies in contradistinction to fleas and some other insects have a prolific intestinal flora which makes it impossible to obtain pure cultures directly from any part of the alimentary canal. The present writer has many times convinced himself of the fruitlessness of such an attempt. The bacteria carried over into the media together with the *Herpetomonads* soon outgrow them and in a short time kill off the flagellates. These protozoa seem to be able to tolerate a certain amount of intestinal flora under intestinal conditions, but soon die out under artificial conditions, i. e., on artificial media upon which bacteria multiply so rapidly.

Franchini and Mantovani took blood from the heart of one of their rats inoculated three and one-half months previously with the intestinal contents of parasitised house flies. Some of this blood was inoculated into the condensation liquid of the N.N.N. medium (1 part) mixed with a 3 per cent. soluton of glucose (4 parts). In about twelve days a pure culture of little organisms appeared having the aspect of anaplasms in stained smears. At autopsy two mice inoculated intraperitoneally with this culture showed a few Leishmaniform parasites in the liver. The authors were unable to propagate their cultures in transplants.

The present writer inoculated intraperitoneally four white and two wild mice, one rat, and one guinea pig with the intestinal contents of flies heavily parasitised with *Herpetomonas muscae-domesticae.* Care was taken to obtain parasites in the flagellated condition and they seemed active and vigorous. The inoculated animals showed no clinical symptoms whatever. Their blood was examined in stained and unstained condition at intervals of a few days, but nothing was found. Two of the white mice were autopsied in one month. All of the organs were normal and no Leishmaniform bodies were visible in the liver, spleen, bone marrow, kidneys, or other organs. One white mouse and one wild mouse were killed after two months. The examinations were entirely negative. One white mouse, one wild mouse, and the rat were sacrificed in three months. The white mouse had pneumonic lungs, but in every other respect the three animals were normal. No parasites were found. Since the examinations of the supposedly susceptible

animals proved negative, and since the guinea pig showed no symptoms nor parasites in the blood, it was not killed and is still alive today, five months after the inoculation. At no time during this period did any temperature develop nor was there any loss in weight.

The negative results obtained by the writer need not reflect on any of the results obtained by Franchini and Mantovani, and suggest that the authors may have dealt with a geographical variety or with a distinct species, although for morphological reasons the European form and the form studied from this locality may be considered identical. It may be suggested that the *Herpetomonads* of house flies in different parts of the world be carefully compared, and also that a careful seasonal study of the forms occurring in one region be made.

Since it seemed impossible to obtain a pure culture of *H. muscae-domesticae* free from bacteria indirectly by the inoculation of higher animals, another method that proved successful was devised. In 1918, while experimenting with grasshopper diseases and strains of d'Herelle's *Coccobacillus acridiorum,** it was found that many species of Acridians developed an immunity toward bacteria. Many Acridians die of bacterial infections annually, but many also recover and become sexually mature. Such recovered hoppers are comparatively immune and this immunity can be demonstrated. Experimentally also, it was shown that this immunity could be produced in healthy non-exposed animals by inoculation with sublethal doses or with killed cultures of various bacteria. The idea suggested itself that it might be possible to inoculate grasshoppers or Locustids with the intestinal contents of flies containing Herpetomonads and bacteria, and perhaps obtain a pure culture of the protozoa in this way.

In the first experiment, fifty large female grasshoppers *(Melanoplus femur-rubrum)* were inoculated with such material. On August 3 the intestines of five heavily parasitised house flies were removed under aseptic conditions and cut up very finely in sterile Locke's solution in order to liberate the Herpetomonads and mince the intestine, so that no large pieces of tissue would be introduced into the hoppers. The hoppers were then held and restrained, so that the inoculation site could be wiped off with alcohol corrosive sublimate mixture. Twenty-five hoppers were then inoculated each with 0.1 c.c. of Locke's solution containing Herpetomonads, bacteria, intestinal cells and contents. Ten hoppers were inoculated into the body cavity on the ventral side between the thorax and abdomen, and fifteen were inoculated in the hind leg joint between the trochanter and femur. Twenty-five uninoculated hoppers were kept as controls. The inoculated and uninoculated hoppers were then placed into separate sterile glass jars with some grass.

* A systematic study of the organisms distributed under the name of *Coccobacillus acridiorum*. d'Herelle. Annals Ent. Soc. America, 1918, vol. xi.

In 48 hours all but four of the inoculated hoppers were dead. The uninoculated ones were all alive. The dead hoppers gave off an odor of putrefaction and were swarming with bacteria. No Herpetomonads were seen. The four inoculated, but live hoppers were also carefully examined. The leg joint between the trochanter and femur of the hind leg was first wiped off with alcohol and then singed. A sharp, sterile capillary pipette was then introduced and some blood removed. Some of this blood was introduced into ordinary culture media to test for bacterial sterility. The rest was examined microscopically for bacteria and Herpetomonads. In none of the four hoppers were bacteria found microscopically, but in three of the animals a few actively moving Herpetomonads were seen. These preparations were stained by Giemsa's method and the flagellates were identical with those introduced. The N.N.N. medium and a variety of other media were inoculated with some of the blood containing Herpetomonads, but no growth was obtained at room or incubator temperature although tubes were kept for two weeks and examined every few days. Some tubes were also sealed in order to produce a lowered oxygen tension. The media previously inoculated with blood to prove bacterial sterility remained sterile.

Blood from the control hoppers was carefully examined for flagellates in exactly the same manner. No bacteria nor protozoa of any sort were found. In the writer's previous experiences with hundreds of hoppers comprising many species, he has never found protozoa in the blood of these animals.

These experiments, therefore, prove that out of a large series of hoppers inoculated with the intestinal contents of parasitised house flies, a small number will survive, will free themselves of the intestinal bacteria in about 48 hours, and will maintain *Herpetomonas muscae-domesticae* for at least 48 hours or more.

Since no growth of Herpetomonads was obtained on the media used, an insect medium suggested itself, but since one can secure so little blood serum or tissue juices from hoppers another insect was used.

The writer was fortunately rearing large numbers of the larvae of the meal moth *(Euphestia küniella)*. About two hundred large meal worms were taken and rubbed up in a mortar until nearly all of the juices were expressed. Twenty-five c.c. of sterile Locke's solution was added at intervals to facilitate the grinding. This material was then strained through a cheese cloth after which it was filtered through paper. The filtrate amounted to 35 c.c. This was then filtered through sterile Berkefeld candles. At the same time some fresh horse serum was filtered through a Berkefeld candle. To 10 c.c. of the diluted insect juices, 30 c.c. of horse serum was added and the two materials mixed. This mixture was then tubed into small tubes, 1.5 c.c. per

tube. The tubes were then put into an inspissator and the temperature raised to 74 C., at which temperature the serum coagulated. The time during which the temperature was raised from 60 C. to 74 C., and lowered again to 60 C. consumed one hour and thirty minutes. The tubes were then removed. Two drops of normal grasshopper blood were then permitted to flow over each slant, after which the tubes were incubated for 48 hours as a test for bacterial sterility. Later the tubes were stored in the refrigerator after being sealed with sealing wax to prevent evaporation.

August 18, fifteen grasshoppers *(Melanoplus femur-rubrum)* and two locustids *(Amblycorypha oblongifolia)* were again inoculated as in the previous experiment. Fifteen hoppers constituted the controls. In 48 hours all the controls were alive excepting two. These two were examined but nothing excepting bacteria found. All the experimental hoppers but one were dead. The two locustids were alive. Blood was examined from the hopper and two locustids and Herpetomonads were found in all three. No bacteria were present, nor did any growth appear subsequently in inoculated media. Some blood from the three positive cases was inoculated into some of the special insect-horse serum medium previously described. Two tubes were inoculated from each animal, sealed with sealing wax and kept at room temperature. At the end of seven days the tubes were examined and a light growth of Herpetomonads was found in the liquid in the bottom of some of the tubes. The growth was found in one tube inoculated with hopper blood and in two tubes inoculated from the blood of one of the locustids. The two tubes inoculated with blood from the other locustid showed no growth. All the tubes were sterile for bacteria. In the tubes in which growth occurred the Herpetomonads were all in the flagellated condition growing in rather dense clumps. No pre- or post-flagellated phases were seen and those present, as stained smears demonstrated, appeared to have been produced by the longitudinal division of pre-existing flagellated forms. When the flagellates were examined in Locke's solution they appeared to be quite vigorous and active, although their activity was somewhat hampered by excessively long and wavy flagella. The flagella at times appeared to be twice the length of the flagella seen in preparations of the Herpetomonads direct from fly intestines. Otherwise, the morphology of the cultivated forms corresponded to the long, lancet shaped forms found in flies. Transfers to fresh insect media were immediately made. In the original tubes the flagellates died out during the course of the next week. After a week the transfers again showed a growth, but not nearly so prolific as in the original tubes. Some material from one of these tubes was inoculated intraperitoneally into two white mice. One mouse was autopsied in four weeks and the other in ten weeks. Absolutely nothing was found.

A second cultural transfer was made, but nothing grew. Examinations every few days revealed a partial recovery of the flagellates put in, but these soon died out and no multiplication occurred. The organisms in the first transfer tubes also died out very soon.

While the examinations were being made, 15 hoppers were inoculated September 5. The results were almost identical with the foregoing. A fairly luxuriant growth was obtained on the first set of tubes. Transfers were made and the resulting growth was weaker. Second transfers were again attempted, but no growth ensued.

SUMMARY

House flies in proximity to cattle were found engorged with cow blood. A close association between the feeding habits of Stomoxys, Hæmatobia and *Musca domestica* was observed. House flies often feed at the punctures deserted by the biting flies. *Herpetomonas muscae-domesticae* was found to be the most prevalent flagellate inhabiting the digestive tract of adult house flies in summer. The number of flies parasitised was large. The greatest degree of parasitism was reached in July and August. The parasitised flies were always caught in cow barns and in horse stables. Flies caught in dwelling houses were not parasitised.

Some morphological details pertaining to the flagellate are discussed and the opinion is expressed that the morphology described is identical with that studied by other workers.

Experimental Leishmaniases or Herpetomoniasis is reviewed and discussed. The writer was unable to produce either with *Herpetomonas muscae-domesticae.*

The view is tendered that probably more than one variety of *Herpetomonas muscae-domesticae* exist and that these varieties may be detected solely on the basis of pathogenic and other physiologic properties.

A special method for the pure cultivation of *Herpetomonas muscae-domesticae* is described. The flagellated form was cultivated and reproduced itself by longitudinal division.

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Explanation of Plate XI

All figures represent *Herpetomonas muscae-domesticae*. × 813.

Figs. 1-4.—Types of mature and maturing flagellates.

Fig. 3.—Herpetomonad showing deeply stained rod, and vacuole at anterior end.

Fig. 5.—Division of flagellum and blepharoplast.

Fig. 6.—Division of trophonucleus and cell.

Fig. 7.—Advanced cell division.

Figs. 8 and 9.—Other morphological types.

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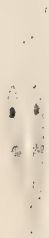

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PLATE XI

THE RAT TAPEWORM, *HYMENOLEPIS DIMINUTA*, IN MAN

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Cases of the occurrence of the rat tapeworm, *Hymenolepis diminuta*, as a parasite of man are of such rarity as to merit special record. Moreover, many important facts regarding the life history of this worm have been brought to light since the comprehensive review by Ransom (1904), and a general discussion of the subject seems desirable.

UNREPORTED CASE

In August, 1921, an infant, nine months of age, was brought to the Miller Hospital Clinic, St. Paul, Minnesota. Her mother stated that the child was suffering from an intestinal upset, with green, foul smelling stools. She stated that the bowel movement contained worm segments. Examination of the stool showed these segments in numbers. In addition to the intestinal upset, the baby had an eczema, which was treated according to the usual way.

Apparently following this visit the baby got very much better and the mother did not return, in spite of the fact that she had been advised of the necessity of placing the baby in the hospital for treatment for the tapeworm. It was some three and a half months later that she decided she wanted the tapeworm taken care of. Her decision was forced by the fact that the child was extremely restless, especially at night, and that she was remaining stationary in weight, in spite of a fairly rational diet. The baby was placed in the hospital and treatment instituted. The treatment was only partially successful, for although about twenty worms were recovered, careful examination failed to reveal any complete specimens, and at the time of writing, a month later, segments are still present in the stool. The results of the treatment have been partially satisfactory in that the child has been much better since the hospital regime. The discharged worms agree in every respect with descriptions and specimens of *Hymenolepis diminuta*, except in scolex characters, which were not available. The child had been breast fed, and reared in a good home, in a cleanly environment. The clue to possible sources of infection was obtained when the mother stated that in mid-summer at the age of seven months it had been allowed to creep on a grassy plot in the yard.

SOURCES OF INFESTATION

Hymenolepis diminuta is the most common of the rat tapeworms. It is reported not only for various species of rats, but also for the common mouse. Few data relative to its frequency in these rodents are available and as is often the case in statements regarding infection, some of the figures are open to the suspicion of being vitiated by examination of animals from centers of infection.

We have found it in 14 per cent. of rats examined in Minneapolis and St. Paul. Moll (1917) reports it in five out of twenty-five examinations at Madison, Wisconsin. Grassi found it in 20 to 30 per cent. of Italian rats. Joyeux states that 18 out of 101 rats captured in Algeria were infested and 15 out of 25 in Salonica. Brumpt found it in 55 per cent. of the Norway rats examined in Paris, but states that it was rare in mice.

The researches of Grassi and Rovelli showed that the cysticercoid of this rat tapeworm developed in the body cavity of a surprising range of meal-infesting insects. This work has been fully reviewed by Ransom (1904) and is very generally cited in the literature. These authors found that development occurred in the larvae and adult of a moth, *Asopia farinalis,* in both nymphs and adults of the earwig, *Anisolabis annulipes,* and in adults of the tenebrionid beetles, *Akis spinosa* and *Scaurus striatus.* Grassi considers that the lepidopter is the normal intermediate host.

With so wide a range of hosts, including two tenebrionid beetles, it would seem probable that the common meal-worm, *Tenebrio molitor,* would serve as an intermediate host of *Hymenolepis diminuta.* With this in mind one of us, and an enthusiastic student, Miss Olga Holie, made numerous attempts to infect these beetle larvae. These efforts were uniformly unsuccessful. We have since noted that this was the experience of Joyeux (1920) with larvae of *Tenebrio molitor,* but on the other hand, he found that adult beetles of this species were the most readily and uniformly infected of any of the numerous forms with which he experimented. We made no effort to infect the adults.

Dr. Nickerson informs us that in attempting to trace the source of a case of infection of a child in Minnesota (Nickerson, 1911), he found that some months previous to the noting of the worms "there had been a pest of myriapods (Julidae) which over-ran the house and were into milk pans and all over." Assuming that the child might have acquired its parasites through the accidental ingestion of one or more of these myriapods, Dr. Nickerson collected living *Hymenolepis diminuta* from rats, and fed them chopped up and scattered on leaves, etc., to young myriapods of the species *Fontaria virginica* and *Julus* sp. In both of these the cysticercoids were formed.

Nicoll and Minchin (1911) found the larval stage of *Hymenolepis diminuta* in about 4 per cent. (8 out of 207) of rat fleas, *Ceratophyllus fasciatus,* examined during the thirteen months. Usually there was a single cysticercoid present, but in some cases there were as many as three.

Then, in order to test their conclusion that the cysticercoids were those of *Hymenolepis diminuta,* a litter of young rats was separated from the mother as early as possible, isolated, and fed on boiled bread and milk. During three months frequent examinations of feces showed them free from tapeworms. Two of the rats were then fed daily on rat fleas mashed up in their food. This was continued for nineteen days, feeding being omitted on three of these. At this time one of the rats died. No ova had been found in its feces but there were present in the alimentary canal five specimens of *H. diminuta* varying in size from 1 cm. up to 40 cm. On the next day numerous ova were found in the feces of the second rat. The check rats of the litter were observed for two months but no tapeworm eggs were found. The experiment was repeated later with similar result, ova appearing in the feces on the nineteenth day. The rat which had survived the first experiment was eventually killed and nine worms were found. The total of 14 tapeworms recovered was the result of feeding 340 fleas, which indicates that about 4 per cent. contained cysticercoids—a result agreeing with the number found in fleas which were actually dissected. From this it was evident that the complete development of *Hymenolepis diminuta* in the definitive host took place in less than three weeks. Calandruccio found ova in his feces fifteen days after he had experimentally swallowed the cysticercoids of this species, so it is evident that the worms mature in from two to three weeks.

The findings of cysticercoids in the bodies of *Ceratophyllus fasciatus* were confirmed by Johnston (1913) in Australia. He also found that the flea *Xenopsylla cheopis* might harbor the cysticercoids.

Joyeux (1920) found these two species of rat fleas so easily infected that he regards them, and the adult *Tenebrio molitor* as the natural intermediate hosts of *Hymenolepis diminuta.* Infection of fleas occurs only during the larval stage of the insects. This relation between the flea and the rat tapeworm is not surprising when it is recalled that the larval fleas develop in the debris and rat feces where rats abound. Joyeux was also able to infect experimentally larvae of the fleas, *Leptopsylla musculi, Pulex irritans* and *Ctenocephalus canis.*

CASES OF HYMENOLEPIS DIMINUTA IN MAN

As already noted, Ransom (1904) has already reported twelve cases of the occurrence of *Hymenolepis diminuta* as a parasite of man. In this number was included the experimental infection of Calandruccio.

To these should be added not only the one herein reported, but also the following 41 cases already noted in the literature, and 7 in the addendum to this article, making a total of 61 infections known for man. This total counts the report of Hoki (1917) as a single case.

Deaderick, 1906 ..... 1 case

A white boy, 8 years old, who had never been outside of Lee County, Arkansas, had a ravenous appetite, slight nausea, and pain in the epigastrium for two weeks. Examination of the feces showed about 10 ova of *Hymenolepis diminuta* in the preparation.

After a saline the night before the boy was given 25 drops of oleoresin of male fern. There were discharged four long fragments, measuring 12, 17, 21 and 25 c.c. (obviously a misprint for cm.) and then shorter fragments from 2 to 7.5 c.c. (cm.). One of the long fragments was expelled by the salts before the male fern was given. Unfortunately no head was found.

Five days later, the feces were examined and no ova found. The father reported entire relief from the mild symptoms present before treatment.

Garrison, 1907 ..... 1 case

Examinations of 4,106 prisoners in the Philippine Islands revealed one case of infestation by *Hymenolepis diminuta*. The host was a Chinese prisoner who was freed before there was opportunity to obtain data, so it is an open question as to whether the infection was imported or contracted in the Philippines.

Condorelli-Francaviglia, 1908 ..... 1 case

We have no data relative to this case other than the bibliographic record. The title indicates that the patient was a young girl who had a concurrent infestation of *Hymenolepis diminuta, Ascaris lumbricoides* and larvae of Calliphora.

Galli-Valerio, 1910 ..... 1 case

A woman in the interior of Brazil, June 10, 1909, in the practice of a Dr. Rondino. No further data.

Nickerson, 1911 ..... 1 case

A case of infection of a child in Minnesota is mentioned in connection with a brief report on the possibility of Myriapods serving as intermediate hosts for *Hymenolepis diminuta* (see below). In a recent letter to us, Dr. Nickerson states that the case was that of a child about two years of age, at Hanley Falls, Minnesota.

Noc, F., 1911 ..... 1 case

Martinique, French West Indies. No data.

Rijo, G., 1911 ..... 1 case

Listed by Joyeux (1920). No data.

Leiper, R. T., 1913 ..... 1 case

Records a case in a child at Grenada, West Indies.

Parodi, 1915 ..... 1 case

Argentine Republic. Listed without data by Joyeux (1920).

Hoki, R., 1917..... (?)

In a Japanese medical journal, Hoki reports on the examination of soldiers from the Loochoo Islands. An abstract in the Tropical Disease Bulletin states that the "author identified an egg which agreed in morphology and measurements with that from a rat (*M. decumanus*) and Braun's illustrations of *Hymenolepis diminuta.* A drawing of the head is somewhat different and is reproduced on account of possible taxonomic value."

Shircore, 1917..... 1 case

In the course of 1,500 examinations of feces of native East Africans at the hospital in Mombasa, of Indians of the Expeditionary Force, and of Washikira Arabs of the Arab Rifles, there was found one case, an Arab, infested by *Hymenolepis diminuta.*

Gonzaga and Carvahlo, 1918..... 16 cases

Sixteen cases out of 2,725 subjects, or 0.58 per cent., State of Sao Paulo, Brazil. Cited by Joyeux.

Acton, 1919..... 8 cases

Two thousand nine hundred and eighty-one routine examinations of Indian members of the Mesopotamian Expeditionary Force revealed 8 cases (0.2 per cent.) of *Hymenolepis diminuta.*

Gedoelst, 1920..... 1 case

That of a Belgian infected while serving in the Belgian Congo on military duty, between 1916 and 1919.

Schwartz, 1921..... 1 case

Before the Helminthological Society of Washington, Dr. Schwartz reported a case of this tapeworm in the child 2½ years of age, in the practice of Dr. C. C. DuBoise of Warsaw, Indiana. Gravid segments had been collected from the stool and it was stated that the child had passed several feet of tapeworm on a previous occasion.

Cort, 1921..... 1 case

In the discussion of the report by Schwartz, Dr. Cort mentions that "Dr. Mallory had found a case of the same sort in Nicaragua." Dr. Cort informs us that he is unable to furnish data as to the age of the patient.

Stiles, 1921..... 3 cases

On the same occasion Dr. Stiles mentioned three unpublished cases of *Hymenolepis diminuta* in man that had come to his attention. In one case, specimens were collected by Dr. Talcott, at Greenwood, Nebraska in 1906, in another by Dr. Constans, at Washington, D. C., in 1911, and in another by Dr. Leonard at Gastonia, N. C. In a recent letter to us Dr. Stiles states that the patients in each case were children aged, respectively, 2 years, 12 years, and 17 months.

AGE DISTRIBUTION

The age distribution of 14 out of the total 54 cases is definitely stated as follows: 9 months, 17 months, 19 months, 20 months, 2 years (4 cases), 2½ years, 3 years, 8 years, 11 years, 12 years (2 cases). Of the remaining 40, three were noted merely as children. A total of 15 were adults, ten of them having been brought to light through the examination of troops. Concerning twenty-two (including Gonzaga's and Carvahlo's 16), we have no available data.

In this connection it is of interest to note that, in as far as published, extensive examinations of troops in this country have not revealed any cases of *Hymenolepis diminuta* infestation. A striking illustration is afforded by Lucké (1919), who in the study of the prevalence of intestinal worms in 35,000 white and colored troops of Camp Zachary Taylor, Kentucky, found no such case, although he did find 238 cases of *Hymenolepis nana.* Kofoid, Kornhauser and Plate (1919) in the course of examinations of 1,200 overseas and 300 home service troops found *H. nana* 7 times (all overseas men) but no *H. diminuta.*

The case which we report holds the record of early infection by *Hymenolepis diminuta,* since infection occurred before the ninth month.

On the other hand, Vacca (1909) has noted infection by the double-pored tapeworm, *Dipylidium caninum,* which also develops in the flea; in an infant of about twenty days. At this time the child began to suffer from an enteritis which was resistant to treatment. Toward the second month, there were found in its dejections three white segments. About a month later, the enteritis continuing, and the infant being restless, and later in a stupor, with an enormous papillary dilation, a physician was consulted. On treatment, at the age of three and a half months, the infant discharged a worm 105 mm. long, without either head or neck. The health of the child was progressively established. The only animal living in the family of the patient was a cat brought into the house six days after the birth of the child, i. e., about twelve days before the beginning of the intestinal troubles. The cat was killed and found to contain 31 specimens of *Dipylidium caninum.* The fleas living in the fur of the cat were also examined; one of them harbored cysticercoids in its body cavity.

Of 76 authentic records of human infestation by *Dipylidium caninum* 23 cases, or 30.26 per cent. of the total were in children under six months of age, while over 65 per cent. were under three years. This great percentage of prevalence of this tapeworm in infants is due to the fact that its development takes place primarily in the cat or dog flea and that babies are very often exposed to possible infection indirectly from pet dogs or cats.

ADDENDUM

Since this paper was presented before the Helminthological Society of Washington there have come to the attention of the senior author seven additional cases of infestation of humans by *Hymenolepis diminuta*. The total figure mentioned in the body of the text has been revised to include these, making a grand total of 61 cases known. The available data regarding the seven cases are as follows:

Chandler (1922) records three not previously reported cases of *Hymenolepis diminuta* observed in the course of hookworm resurvey work in Louisiana and in Georgia. These were:

1. Boy, aged 7, white, Mansfield, De Sota Parish, La., July 30, 1921.
2. Boy, aged 13, negro, Clontierville, Natchitoches Parish, La., June 19, 1921.
1. Boy, aged 7, white, Mansfield, De Soto Parish, La., July 30, 1921.

Dr. Chandler also informs us in correspondence that he had omitted from his summary a case reported by De Bugs and Dwyer, 1919.

Dr. C. W. Stiles has kindly furnished us data regarding a fourth unreported case in the collections of the U. S. Public Health Service. This specimen was collected by Dr. Hopkins at Richmond, Va. The patient was a child 2 years old. It should be noted that through a misunderstanding this child's case was confused with that submitted to the Public Health Service by Dr. Talcott. The data with the latter specimen made no mention of the age of the patient.

Through the courtesy of Dr. D. M. Molloy, director for Nicaragua of the work of the International Health Board, I have more detailed information concerning the case already noted and data concerning two additional cases, not included in the foregoing summary. The data are as follows:

Case No. 1 (1918).—Female child, 3½ years old, resident of Masatepe, department of Carazo. Under treatment for hookworm infection expelled two parasites which were classified by technician who strained the stools as *Hymenolepis nana* (which is quite common in Nicaragua). No data as to length of time infestation continued or the source of infestation. Parasites were later identified by me as *Hymenolepis diminuta*. Two parasites were expelled, one of which measured about 30 cm. in length (preserved in 5% formalin), and the other measured about 40 cm. These specimens were lost.

Case No. 2 (1919).—Female child, 9 years old. Four parasites were expelled after the taking of a purgative prescribed by a physician, who sent the specimens to the laboratory for identification. These specimens were sent later to the Naval Medical School in Washington, where the identification was verified and where the specimens now are. No data as to probable source of infection or length of time it had persisted. Child was lost sight of. Resident of the "sierras" of the department of Managua.

Case No. 3 (1920).—Female child 7½ years old, resident of the city of Managua. Diagnosis made on examination of feces for ova of intestinal parasites, the characteristic eggs of *Hymenolepis diminuta* being found, together with eggs of hookworm, Ascaris and Tricocephalus. The patient never returned to

the dispensary for treatment, hence no data of the source of infection or its probable duration collected. We are making an effort to locate this patient for collecting data and specimens.

Thus there are known at least 61 cases of infestation of man by *Hymenolepis diminuta*. These are distributed geographically as follows: Brazil, 19; United States, 16; India, 8; Italy, 7; Nicaragua, 3, and one each for Argentine, Belgium, Cuba, East Africa, Grenada, Japan, Martinique, and the Philippines.

It is quite probable that in extensive routine examinations the eggs of *Hymenolepis diminuta* have been at times mistaken for those of *Hymenolepis nana* and that the above figures should be considerably higher. On this point Dr. Molloy writes "This would naturally be the case with untrained technicians, who make their diagnoses with the aid of charts, and who have relatively little knowledge of the cestode parasites."

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A NEW MYXOSPORIDIAN PARASITE OF THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS* *

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During several summers the writer has been making a somewhat extensive study of the myxosporidian parasites of fishes from the Mississippi River at Fairport, Iowa. In the course of these investigations a number of channel catfish, *Ictalurus punctatus,* have been examined for parasites and one individual was found to be badly infected with a new species of Henneguya. This species is notable for a number of exceptional and interesting characteristics, and for that reason it has been thought advisable to publish an account of it in advance of the main results of the investigations.

This species, for which I propose the name *Henneguya plasmodia.* occurs in the epithelium of the gill filaments as a small amoeboid plasmodium. It never forms cysts as do the other Myxosporidia occurring on the gills but retains its amoeboid form throughout life. It is apparently not common since it was found in only one fish † out of 29 examined. In this case, however, it was present in enormous numbers.

Henneguya plasmodia is too small to be visible with a hand lens but when the gills were examined under a magnification of about 150 diameters large numbers of small amoeboid parasites could be easily distinguished. They were so numerous that often six or eight parasites were in the field at one time. At first it was thought that the parasites were simply clinging to the surface of the gills but a later study of sectioned material showed that they were in reality interspersed among the epithelial cells. All stages from small vegetative trophozoites to adult sporulating forms were abundant.

The trophozoites resemble the coelozoic or "free" forms rather than the tissue parasites. Both vegetative and sporulating trophozoites are very irregular in shape with several short, conical pseudopodia by means of which they move slowly about among the epithelial cells. The trophozoites are colorless and distinctly granular with usually no trace of a distinct ectoplasmic layer, although a few individuals were observed

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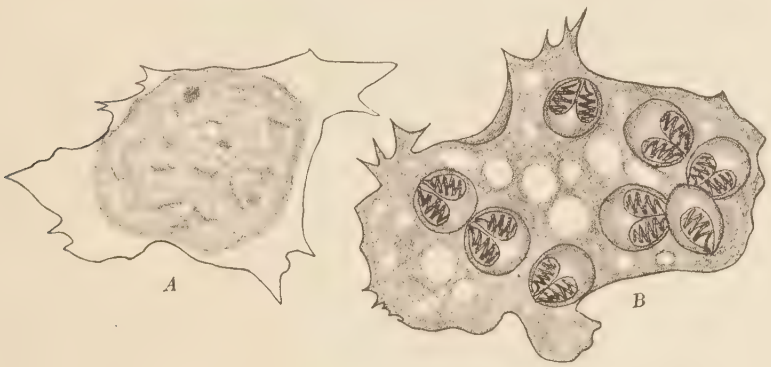
† This fish was one of a number which had been used in experiments on mussel propagation during the course of which they were held for several weeks in a small cement pond. Seven other fish used in the same experiment were examined with negative results.

in which the ectoplasm was quite noticeable. Trophozoites which had been removed from the gills with a small amount of epithelium and placed under a sealed cover-glass for 24 hours showed almost without exception a well developed layer of hyaline ectoplasm (Fig. A), although in all other respects they appeared perfectly normal. However, this fact is of no particular significance since it is quite common for trophozoites of the coelozoi Myxosporidia to show a more distinct ectoplasmic layer after being kept on a slide for a time. The trophozoites often contain a few fat globules but these are never abundant and may be entirely absent. They are usually more or less distinctly vacuolated, in some cases the vacuoles being quite conspicuous (Fig. B). Aside from the presence of spores there is very little difference in the appearance of the vegetative and sporulating trophozoites, except that



A, Vegetative trophozoite which had been kept on a slide for several hours. The hyaline ectoplasmic layer is very distinct. × 1600.

B, Living sporulating trophozoites as they appear in the gill epithelium. × 1600.

the latter are naturally somewhat larger. The average diameter of the sporulating trophozoites, based on measurements of both fresh and preserved material, is about 25μ to 30μ.

The true relation of the trophozoites to the epithelial cells can best be seen in sections. Figures 1 to 5 show vegetative trophozoites in various stages of development. The smaller individuals may sometimes actually penetrate the epithelial cells as shown in figure 1 but this appears to be exceptional. Usually they are clearly located between the cells (Fig. 2). Apparently the trophozoites may actually destroy the epithelial cells since it is not rare to find the nuclei of such cells in direct contact with the trophozoites (Figs. 3 to 5). The smallest trophozoites observed contained several nuclei which, as in other species

of Myxosporidia, were plainly of two types, "vegetative" and "generative." The generative nuclei are usually surrounded by a thin layer of modified endoplasm forming distinct generative cells (Figs. 2, 4 and 5).

As the trophozoites enlarge they are in most cases covered externally by only a single layer of epithelial cells which become so stretched as to form a very thin covering (Figs. 3, 4 and 6). Occasionally this covering may entirely disappear (Fig. 7) so that the trophozoites are in effect simply attached to the external surface of the epithelium. Such instances are, however, not common since in the great majority of cases the trophozoites are protected by a very thin layer of modified epithelial cells. Since this layer is perfectly transparent the trophozoites in fresh material appear to be clinging to the surface of the gills.

While no trophozoites were observed which appeared to be dividing it is probable that they do multiply by plasmotomy or schizogony since the presence of the parasites in such enormous numbers would be difficult to explain in any other way. Sporulation was the only method of reproduction observed, most of the trophozoites containing spores in various stages of development (Figs. 6 and 7). The number of spores observed in a trophozoite varied from 2 to 8.

The spore when viewed from above appears approximately circular (Fig. 8) with a long slender process extending from the postcapsular side. This postcapsular process, which is characteristic of the genus Henneguya, does not taper gradually toward the free end as in most cases but is the same diameter throughout except near the tip where it tapers rapidly to a point. The spore is only slightly compressed parallel to the sutural plane and when viewed from the side appears pyriform, tapering slightly toward the capsular side (Fig. 9). The sutural ridge is distinct but not prominent. The capsules are large and conspicuous, the enclosed filament being easily seen in the fresh spore. The sporoplasm, which can be readily distinguished, is very finely granular throughout. No iodophile vacuole could be demonstrated in the fresh spore by treatment with iodine, but in mature spores in sectioned material a clear vacuole could be easily distinguished in the sporoplasm. The length of the spore exclusive of the postcapsular process is 6μ while the process is about 15μ long, giving a total length of about 21μ. The width of the spore is about 7μ to 8μ. The capsules are 4.5μ long by 3μ broad.

The postcapsular process can only be distinguished on spores which have been liberated from the trophozoite and then often with great difficulty owing to its great transparency. While enclosed within the trophozoite no trace of the process can be made out (Fig. B) even in the case of fully matured spores.

The spore is quite similar to those of *H. macrura* Gurley and *H. brachyura* Ward but the vegetative stages are very different. *H. macrura* forms cysts the size of a pinhead in the subcutaneous connective tissue (Gurley, 1894) of the head of *Hybognathus nuchalis*. The postcapsular process of the spore has a very different structure than that of typical species of Henneguya. According to Gurley it is completely dissolved by sulphuric acid and becomes invisible when the spore is mounted in balsam. It consists of a single long median piece with two short lateral processes where it joins the main body of the spore.

The spore of *H. plasmodia* even more closely resembles that of *H. brachyura* (Ward, 1919). The dimensions are about the same but the sutural ridge is not as well developed as in *brachyura* and it entirely lacks the folds so characteristic of that species. Owing to lack of material Ward was unable to make careful study of the structure of the postcapsular process, but emphasizes the fact that it appeared to be entirely different from the ordinary bifurcated type. He found that in Giemsa's solution the shell proper stains a clear blue while the tail takes on a beautiful pink color. In dried smears of *H. plasmodia* stained with Giemsa's solution the shell proper also stains a light blue but the postcapsular process is entirely unstained.

As in the case of *H. macrura* the most striking difference is in the vegetative stages, *H. brachyura* forming small rounded cysts on the fin rays of *Notropis anogenus*. This character alone is sufficient to differentiate the present species from *brachyura*, although they are evidently closely related. The structure of the postcapsular process in *H. macrura, brachyura* and *plasmodia* is so different from the typical bifurcated process in Henneguya that these three species should probably be included in a separate genus.

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Explanation of Plate XII

Figures 1 to 7 are from sections through the gills of *Ictalurus punctatus*. Only the epithelial cells with the enclosed parasites are shown.

Fig. 1.—A small multinucleated trophozoite (tr.) can be seen within an epithelial cell. × 1450.

Fig. 2.—Vegetative trophozoite at a somewhat later stage than figure 1. Several generative cells can be distinguished. × 1450.

Figs. 3 to 5.—Vegetative trophozoites at a considerably later stage than the preceding figures. In figure 3 a large clear vacuole is present in the trophozoite. Note that the parasites are covered externally by only a single layer of flattened epithelial cells. × 1300.

Fig. 6.—Sporulating trophozoite. The irregular spaces between the trophozoite and the surrounding epithelial cells is undoubtedly due to shrinkage. × 1300.

Fig. 7.—Large sporulating trophozoite which has broken through the external covering of epithelial cells. × 1300.

Fig. 8.—A fresh spore viewed from above. × 2170.

Fig. 9.—A fresh spore viewed from the side. × 2170.

PLATE XII

A NEW RAT TAPEWORM, *SCHIZOTAENIA SIGMODONTIS,* FROM NORTH AMERICA

Asa C. Chandler

and

Charles L. Suttles

(Contribution from the Biological Laboratory, Rice Institute, Houston, Texas.)

During the summer of 1920, while the writers were engaged in an examination of rats of various kinds for evidence of plague infection, a considerable number of specimens of the East Texas cotton rat, *Sigmodon hispidus texianus,* were brought to the laboratory for examination, and were found to harbor a tapeworm of the family Anoplocephalidae which proved to be a new species.

The East Texas cotton rat is an extremely common rodent throughout the humid portion of eastern Texas, and is a sub-species of the common cotton rat of the Southeastern States. It lives around the edges of fields and woods, making runways under dense vegetation or brush, and feeding on vegetation almost exclusively, devouring stems, leaves, seeds and fruits. No evidence of insect remains were found in numerous stomachs examined. Of 96 rats examined, 73, or 75 per cent., were found to be parasitized by the tapeworm here described. The number present in each rat varied from one to thirty, six to ten being the most frequent numbers found.

The worm is a typical representative of the genus Schizotaenia as characterized by Douthitt (1914), except with respect to the uterus, which will be discussed later. In general morphology and anatomy, again excepting the uterus, it seems to come nearer to *Schizotaenia americana* of porcupines than to any other described species, though in the branched excretory system and relatively enormous cirrus and cirrus pouch it approaches *Schizotaenia anoplocephaloides,* described by Douthitt from gophers, *Geomys breviceps,* at Norman, Oklahoma. It is interesting to note in this connection that the only other helminth found in the cotton rat is *Protospirura ascaroidea,* hitherto described only from the same species of gopher, and from the same locality, as *Schizotaenia anoplocephaloides.* Gophers and cotton rats live in much the same way, and it is not surprising to find this similarity in their parasites. The occurrence of a Protospirura in both may be taken as strong circumstantial evidence that both eat insects with the vegetable diet, since the entire group of nematodes to which Protospirura belongs are, so far as known, dependent on invertebrates as intermediate hosts.

The worm here described, which is named *Schizotaenia sigmodontis,* is very variable in size, even among specimens from an individual host.

Mature strobilae range from 21.5 mm. to 65 mm. in length, but the smallest specimens still have a rounded sterile terminal segment, reminiscent of the button on a rattlesnake's rattle. The longest specimens, on the other hand, have an unusually large number of ripe proglottids adhering. Average specimens measure from 30 to 50 mm. in length. The number of proglottids in mature specimens varies from 70 to 90.

With the exception sometimes of one or two terminal segments, the proglottids are all wider than long, those at the middle of the strobila about five times as wide as long. The worm rapidly increases in width behind the head (Fig. 1), and reaches a maximum width of from 2.5 to 3.5 mm. Segments in the middle of the strobila are from 0.45 to 0.65 mm. in length, but back of the middle the proglottids become longer and narrower, those near the end sometimes measuring a little over one half the width of the middle proglottids, and nearly four times their length. The segments project considerably at their posterior margins (Fig. 1), but overlap each other only a little (Fig. 3, *O.*) The genital pores alternate regularly with few exceptions; they are placed near the middle of the lateral margin of the segment and are directed posteriad. The very long and conspicuous cirri, which constitute the most striking feature of the worm, can usually be seen protruding from pores in the posterior portion of the strobila.

The scolex (Fig. 2) is also variable in size, but in a majority of specimens is 0.36 to 0.45 mm. in diameter and about one-half as long; it is not sharply defined from the neck. The very muscular suckers are about 0.16 mm. in diameter. There is no evidence of a rostellum of any kind.

Segmentation begins about 0.6 mm. from the anterior end, thus leaving a short unsegmented neck, unlike other members of the genus. The beginnings of the genital ducts can be faintly observed in very early segments. The vagina and sperm ducts become differentiated out of the common foundation in about the sixteenth segment (2 mm. from the anterior end), and the beginnings of the female genital glands appear at about the same time. The cirrus pouch, cirrus and genital cloaca reach a remarkable degree of development (Fig. 5). The pouch reaches a length of 0.6 mm., about one-fifth the width of the proglottid, with a diameter of 0.19 mm. It has a heavy coat of outer longitudinal and inner circular muscle fibres. The coat of circular muscles is thickened into a distinct sphincter which partially divides the pouch into proximal and distal portions (Fig. 5, *s.m.*). The cirrus becomes greatly elongated, and an enlargement of the seminal vesicle develops in the cirrus pouch behind it, occupying a variable amount of space according to its degree of distension with the seminal fluid (cf. Figs. 4, 5 and 8). The genital papilla, as can be seen from figures 5 and 8, is the everted or partially

everted genital cloaca; it can be protruded 0.38 mm. beyond the margin of the proglottid. The extremely elongated cirrus can be extended 0.75 mm. beyond the genital papilla. When withdrawn into the genital papilla and cirrus pouch, the lumen of the cirrus and a short crown over its opening are covered with numerous fine spines. When protruded, the spines come to lie on the outside of the cirrus, but are very easily broken off, as Douthitt has pointed out in the case of other Schizotaeniae. Probably the spines are lost as the result of copulation, since the cirrus, even in very carefully handled specimens, often appears quite smooth on its exposed surface. The condition shown in Fig. 5 is probably due to a more extensive protrusion than had previously occurred, resulting in the spines near the tip not having been rubbed off.

The elongated seminal vesicle is only slightly convoluted (Figs. 4, 7) and passes toward the anterior margin of the proglottid; it becomes abruptly narrowed and turns postero-medially as the vas deferens, dividing into right and left vasa efferentia. In segments where the copulatory apparatus is evidently in functional condition, the seminal vesicle is often greatly distended with the seminal fluid. The testes, about 70 in number, are arranged in a band extending almost from one excretory canal to the other, toward the posterior side of the segment (Figs. 3, 4). The majority of the testes lie on the aporose side of the genital glands, but some lie dorsal to the glands themselves and about eight or ten are on the pore side. The testes appear to be at the height of their development, with maximum diameters of about 60μ to 85μ, in the segments in which the uterus begins its development, but the copulatory apparatus remains in a functional state almost if not quite to the end of the strobila, and the seminal vesicles continue to contain seminal fluid after the eggs are developed.

The female reproductive system, consisting of ovary, yolk gland, shell gland, seminal receptacle and vagina, becomes fully developed in about the forty-eighth segment (Fig. 4), 12 to 15 mm. from the anterior end. The vagina opens into the genital cloaca just anterior and ventral to the opening of the male system (Fig. 4, 5, 6, 7). There is usually a dilation of the tube into a pouch of variable size, shape and position, which is sometimes larger than the more regular seminal receptacle which follows it (Figs. 6 and 7); often these two pouches are connected by a very short, narrow twisted duct. The receptacle is very large and conspicuous, reaching a length of 0.3 mm. and a width of 0.15 mm. A very delicate duct passes from it in a medio-posterior direction and branches into a duct leading to the shell gland and vittelarium, and a short oviduct (Fig. 7). The large shell gland lies dorsally over the yolk gland, and is about 0.15 mm. in diameter. The ovary and yolk gland are only a little displaced toward the pore side (Figs. 3 and 4); the ovary surrounds the bilobed yolk gland in a

crescentic manner. The yolk gland is bilobed, the median lobe being larger, and each lobe is subdivided into small radiating lobules, as described by Douthitt in *S. americana.* In cleared unstained segments in which the uterus is undergoing development, the yolk gland appears filled with a dense yellowish substance.

The uterus of *S. sigmodontis* is of particular interest. The development of the uterus of the genus Schizotaenia has long been a matter of doubt. Stiles (1897) describes the uterus in *Bertia americana* and *Bertia americana leporis,* both later referred to Schizotaenia, in a very indefinite manner; in the former worm he states that the development of the uterus could not be followed in detail and in the latter that the uterus spreads from the female glands. Janicki (1904) separated the genus Schizotaenia from Bertia (later changed to Bertiella) largely on the basis of the uterus. In Bertiella the uterus develops as a transverse tube with anterior and posterior egg pouches, whereas Janicki described the uterus in Schizotaenia (*S. hagmani*) as developing by a horizontal splitting of the parenchyma from which the uterus extends laterally by means of out-pocketings, and into the rest of the proglottid by a complicated "spaltenwerk." Douthitt, in 1914, described the uterus of *S. americana* as first recognizable as an extensive sheet of tissue which thickens in definite lines and may represent a degenerate reticulum. In *S. anoplocephaloides* he described it as first appearing as a sheet of cells dorsad of the ovary and ventrad of the testes, with circular thickenings around the margins of the testicular fields connected by a transverse thickening, and with a network of less conspicuous strands, again suggesting a degenerate reticulum. The transverse and circular bands develop into canals and the eggs pass by way of the transverse canal to the circular canals. The latter expand centrad and the transverse canal anteriad, until the cavity of the uterus becomes one continuous sac. The extension from this stage is by a regular out-pocketing. Douthitt believes that the difference in the description of the uteri given by himself and by Janicki is one of interpretation rather than of structure.

Schizotaenia sigmodontis, in spite of its evident close similarity to *S. americana* and *S. anoplocephaloides* in other respects, differs absolutely in the mode of development of its uterus. In this species the ovary itself becomes the central portion of the uterus and radiating out-pocketings make their appearance (Fig. 6) from the periphery of the ovary, not only laterad and anteriad, but dorsad and ventrad also. The radiating branches rapidly fill up the middle field of the proglottid, with the exception of the space occupied by the seminal receptacle, shell gland and testes. The out-pocketings are never empty tubes but seem rather to be formed in consequence of pressure exerted by the rapidly developing and enlarging ova.

It is difficult to correlate this unique type of uterine development with the types described in other Schizotaeniae, but a suggestion may be made. Douthitt believes that the sheets of uterine tissue found in Schizotaeniae represent degenerate reticula. In *S. anoplocepholoides* only the peripheral portions of the sheet develop into canals; the rest of the sheet is nonfunctional. In *S. americana* and *S. variabilis* there is no evidence that any part is functional. Douthitt was unable to follow the uterine development in detail in either species beyond the sheet of tissue which he took to represent a degenerate reticulum, and jumps, in his description, from this stage to the fully developed uterus. It is possible that in these forms the uterus develops as it does in *S. sigmodontis*. If the reticulum should entirely degenerate, the out-pocketing which in *S. anoplocephaloides* arises from the periphery of the part which remains functional would naturally arise from the periphery of the ovary. Possibly the splitting of the parenchyma described by Janicki in *S. hagmani* results from an overdistension of the ovary, which may subsequently burst, the out-pocketing at the edges of the split being developed as the natural response on the part of the parenchyma to the presence of ova. If this is a correct interpretation, *S. hagmani* may be looked upon as illustrating an intermediate stage between *S. anoplocephaloides* and *S. sigmodontis*.

The eggs (Fig. 9) are globular and possess three envelopes. The rather heavy outer shell has a diameter of from 47μ to 53μ. The delicate middle membrane is oval, 33μ by 27μ; the inner membrane immediately surrounds the oncosphere and possesses a typical pyriform apparatus. The oncosphere is 16μ to 18μ in diameter and not quite spherical. The pyriform apparatus is 10μ in length. Apparently the eggs are not normally shed from the proglottids within the body of the host, since they have never been found in the centrifuged feces of infected animals.

The excretory system in *S. sigmodontis* consists of a pair of slender thick-walled dorsal vessels and a pair of very spacious ventral vessels connected by broad transverse tubes, and by a system of anastomosing branches (Fig. 8) more extensive than in other species of Schizotaenia. The longitudinal nerves do not show clearly except near the scolex, where they can be seen lying considerably laterad of the excretory canals. The latter cross the reproductive tubes ventrally. Calcareous corpuscles are present and moderately abundant. Following is a diagnosis of the species:

Schizotaenia sigmodontis, n.sp., Chandler and Suttles, 1922

Diagnosis: Strobila 21.5 to 65 mm. long by 2.5 to 3.5 mm. in maximum breadth, segments, except sometimes terminal ones, broader than long. Proglottids 70 to 90. Scolex unarmed, about 0.38 to 0.45 mm. in diameter, about half this length, not sharply demarcated from

the neck; suckers 0.16 mm. in diameter. Strobilization begins about 0.6 mm. from anterior end. Genital pores regularly alternate, near middle of lateral margins of segments. Cirrus very long, spinous; cirrus pouch large, containing enlargement of seminal vesicle; latter, medial of cirrus pouch, slightly convoluted. Testes about 70 in number, 60μ to 85μ in diameter, in posterior band in median field, more numerous on aporose side; female genital glands slightly displaced toward pore side; ovary crescentic; yolk gland bilobed with radiating lobules; shell gland large; uterus develops as radiating out-pocketings from ovary itself, eventually occupying entire median field as coarse anastomosing branched pouches; ova globular, with three membranes and pyriform apparatus; outer shell 47μ to 53μ in diameter, oncosphere 16μ to 18μ in diameter, pyriform apparatus 10μ long. Calcareous corpuscles present.

Host: Cotton rat, *Sigmodon hispidus texianus*. Life history: Unknown. Types sent to U. S. National Museum.

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Explanation of Plate XIII

Abbreviations Used

cl cloaca, *ct* loose connective tissue, *gp* genital papilla, *o* area of overlapping of segments, *od* oviduct, *ov* ovary, *sg* shell gland, *sm* sphincter muscle, *sp* spines of cirrus, *sr* seminal receptacle, *sv* seminal vesicle, *t* testes, *u* uterine out-pocketings, *v* vagina, *yg* yolk gland.

Fig. 1.—*Schizotaenia sigmodontis*, mature worm, showing protruded cirri and sterile terminal segment. × 1.5.

Fig. 2.—Scolex, neck and anterior proglottids. × 40.

Fig. 3.—Young proglottid, the 36th, showing reproductive organ in early state of development. × 30.

Fig. 4.—Older proglottid, the 47th, just prior to development of uterine out-pocketings, showing fully developed female reproductive organs; testes well developed, but copulatory apparatus not yet functional. × 30.

Fig. 5.—Copulatory apparatus and vagina. × 65.

Fig. 6.—Fifty-second proglottid, showing beginning of development of uterus by radiating outpocketings from ovary. × 30.

Fig. 7.—Left half of mature proglottid showing relationships of genital and excretory ducts as seen from dorsal side. × 30.

Fig. 8.—Mature proglottids showing branched excretory system. × 10.

Fig. 9.—Uterine ova. × 400.

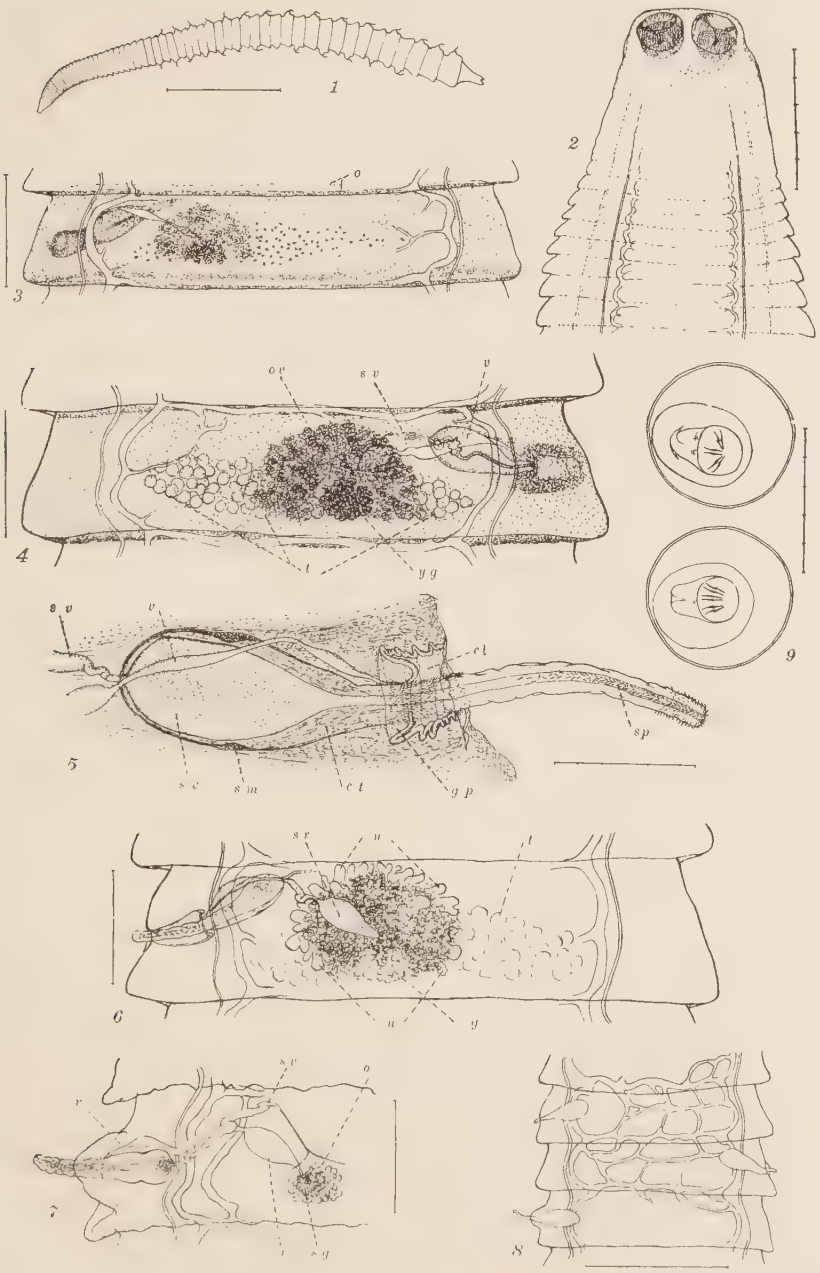


PLATE XIII

NOTES ON A MICROSPORIDIAN PARASITE OF A NEMATODE*

R. Kudo and D. C. Hetherington

Little is known concerning the parasites of the nematode. Up to the present five authors have noted what they regarded as parasites in *Ascaris mystax* of the cat. In the course of study on this nematode, Bischoff (1855) found in the reproductive organ peculiar bodies which he was inclined to regard as spermatozoa. These bodies were yellowish, sharply-contoured and highly refractive, measuring 7.7μ by 4.4μ. Munk (1858) noticed similar bodies in the same organ of the same host. Although these bodies varied from 4.2 to 5.9μ in length and 1.7 to 2.1μ in breadth, the author held them to be identical with those observed by Bischoff and considered them as parasitic algae, stating that "die ovalen Körperchen, wo sie in den Geschlechtskanälen der Männchen vorhanden sind, die Entwicklung der Samenkörperchen und dadurch mittelbar die Befruchtung und weitere Entwicklung der Eier verhindern."

Keferstein (1862) recognized apparently a similar parasite in the intestine and the reproductive organs of this nematode and believed it to be a fungus, naming it *Mucor helminthophthorus*. The oval spores measured 4 to 5μ long by about 2μ broad. Frequently they were surrounded by a membrane and formed a spherical body of about 20μ in diameter. He found a fungus typical to the genus Mucor in the host intestine, which varied from 4 to 20μ in diameter, and considered that the spores enclosed in a membrane were produced by the fungus. Judging from his drawings which were magnified 590 diameters and from the fact that he did not see the spherical bodies containing spores attached to the fungus, it is open to question if the stages described by him belonged to a single form of microorganism.

Lutz and Splendore (1908) described a microsporidian under the name of *Nosema mystacis* which was found parasitic in the intestine and the reproductive organs of *Ascaris mystax* of Brazilian cats. The spores were regularly oval in shape, refractive and showed a round vacuole at the posterior end, measuring 4 to 4.5μ long by 2 to 2.5μ broad. The true nature of the parasites quoted here is unknown. The microsporidian designation of the parasite by the last named two authors is open to discussion since their description of the form is very incomplete.

While examining *Protospirura muris*, a parasitic nematode of the common house mouse, *Mus musculus*, our attention was called to an

* Contributions from the Zoological Laboratory of the University of Illinois. No. 198.

apparent protozoan parasite in the epithelial cells of the intestine. A study of the spores in fresh as well as stained condition and under pressure revealed the fact that the protozoan was a microsporidian for which the name *Thelohania reniformis* nov. spec. is proposed. In view of the circumstance that this is a case of true microsporidian infection in a nematode, it seems worth while to state our observations upon it briefly.

The host nematodes were found in the stomachs of the common house mice caught in the stables and the dairy barns of the University of Illinois at Urbana. The protozoan was found in the epithelial cells of the intestine of the host worm throughout its entire length save the very extremities and isolated spores as well as those still enclosed in the sporont membrane were also noticed in the lumen of the organ. No other organs of the host nematode showed any degree of infection by the parasite, although the body and particularly the reproductive organs were searched for evidence.

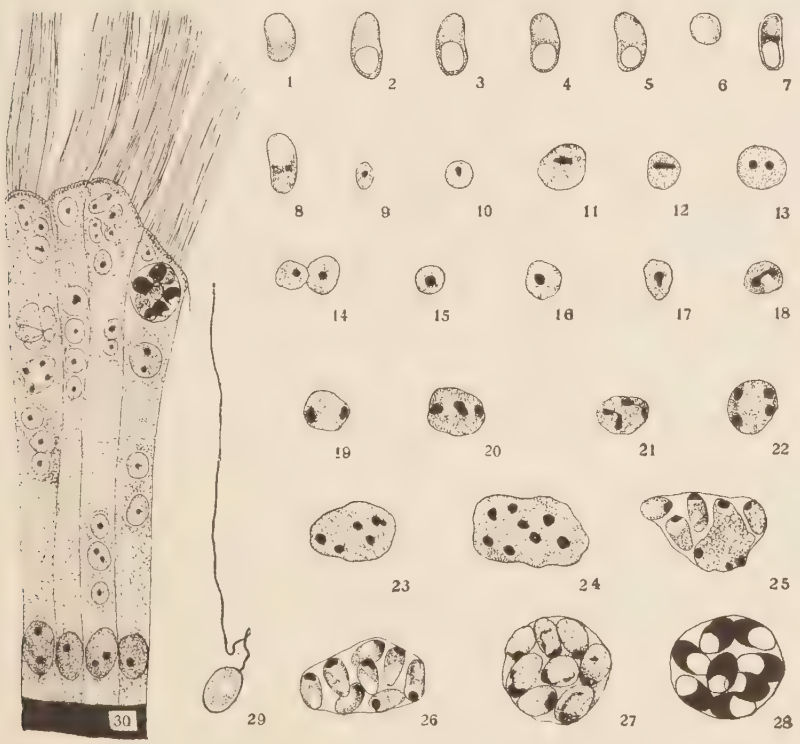
The protozoan was studied in fresh as well as stained smears and in sections. For fixation, sublimate-alcohol-acetic acid mixture, Carnoy's or Fleming's fluid, was used, while Dobell's alcoholic iron hematein, Heidenhain's iron hematoxylin or Giemsa's solution was used for staining. For the demonstraton of the polar filament of the spore, Kudo's method (Kudo, 1920) was employed.

THE SPORE

The spores are kidney-bean shaped (Figs. 1-5) in front view; circular in cross-section (Fig. 6). The spore membrane is comparatively thin and therefore the spores are not so refractive as those of *Nosema bombycis, N. apis, Thelohania opacita* and others. Under an immersion objective the contents of the spore are found to be differentiated into two regions: near one of the extremities, which is frequently more rounded than the other, there is to be seen a rounded clear area, while the remaining portion is finely granulated. The spores measure 3 to 4μ (3.4μ average) long by 1.5 to 1.8μ broad. When a fresh spore is subjected to the action of mechanical pressure (Kudo, 1920: 87), the polar filament becomes extruded. It is comparatively short and thick, measuring 45 to 55μ long in an apparently fully extruded state (Fig. 29).

When the spore is fixed and stained, the clear space mentioned above remains unstained. Along the inner margin of this space, the cytoplasm seems to be accumulated in a zone in which one or two deeply stained nuclear grains are located. The space between this and the other extremity of the spore is occupied by a less deeply stained cytoplasmic mass (Figs. 7, 8). The structure of the spore mentioned here differs from that of *Thelohania magna* (Kudo, 1921), but is somewhat similar to that of *Nosema bombycis* or *Nosema baetis* (Kudo, 1921).

Early stages of the schizogony could only be studied in carefully made section preparations. The stages described here were observed exclusively in the epithelial cells of the host intestine such as shown in figure 30. The youngest schizont is a small oval or rounded mass of finely granulated cytoplasm containing a single nucleus (Fig. 9) and is embedded in the cytoplasm of the host cell. As it grows in size, there becomes visible a narrow clear space between it and the surrounding cytoplasm (Fig. 30). The nucleus is a deeply staining chromatin



Thelohania reniformis nov. spec. Figs. 1-6, fresh spores. Figs. 7-8, stained spores (smears). Figs. 9-14, stages in schizogony (sections). Figs. 15-28, stages in sporogony (sections). Fig. 29, a spore mechanically pressed and stained after Fontana. Fig. 30, four infected epithelial cells of the host nematode (section). Figs. 1-29, × 2200; fig. 30, × 1560.

granule as is ordinarily observed in the schizont of many other Microsporidia. The schizont multiplies in number by binary fission (Figs. 10-14); the nuclear division is amitotic. In all the worms which we examined, we have not seen the condition commonly found in other Microsporidia where the schizogony is repeated rapidly and actively so

that the infected host cells usually become completely filled with the protozoan and even become deformed due to the enormous number of the parasites present in them. Schizogony in the present species is not very active and a slow multiplication by binary fission seems to be the only division process. There seem to exist some inhibiting factors counteracting the activity of the parasites.

The schizonts become the sporonts. Both the nucleus and the body become larger at the expense of the host cells. The cytoplasm of a sporont is more or less vacuolated. The nucleus divides three times, producing eight daughter nuclei and the cytoplasm of the sporont becomes divided into eight sporoplasts (Figs. 15-26). Each sporoblast develops into a spore (Figs. 27, 28). The membrane that surrounds the eight spores thus formed is distinctly seen even after the spores are fully matured.

Since previous authors have seen only stages which appear to be spores of organisms of doubtful nature from *Ascaris mystax,* we are unable to compare them with the forms we found. As the dimensions of the spores of the present form are however entirely different from any of the forms observed in the nematode of the cat and furthermore as the species under consideration has been found in the nematode of the mouse, we believe that we are justified in maintaining the species as hitherto unrecorded. The nucleus of the host cell infected by the parasite is similar in its location and structure to that of the uninfected host cell. The parasite does not seem to cause any pathological changes upon the host organ.

SUMMARY

The records of the parasites of nematodes are reviewed.

A new microsporidian, *Thelohania reniformis,* parasitic in the epithelial cells of the intestine of a nematode, *Protospirura muris* parasitic in the common house mouse, *Mus musculus,* is described.

The schizonts multiply by a binary fission. The sporont develops into sporoblasts and finally into eight spores.

The nucleus of the host cell is not affected by the infection.

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OBSERVATIONS ON THE POISONOUS NATURE OF THE WHITE-MARKED TUSSOCK-MOTH

(HEMEROCAMPA LEUCOSTIGMA SMITH AND ABBOT) *

By Harry H. Knight
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It seems to be the generally accepted opinion among entomologists and others, that larvae of the white-marked tussock-moth are non-poisonous and do not produce toxic reactions when accidentally brought in contact with the human skin. Some recent experience with this insect gives rather definite indications regarding its poisonous nature and opportunity is now taken to record certain observations.

During August, 1921, the writer had occasion to handle and sort a large number of cocoons of *Hemerocampa leucostigma* (= *Orgyia leucostigma*). Mr. Arthur Hertig did the work of collecting the tussock-moth cocoons from the trees on the Minnesota University campus but on the second day he had to give up this work and consult a doctor, for what we supposed must be a severe case of hives. His face, neck, and arms were covered with numerous swellings, resulting in much pain and some fever. The physician was unable to diagnose the trouble as hives, nor in fact was he able to give any good reason for this particular form of dermatitis. The doctor remarked that he had observed perhaps three other cases of a similar nature for which he was unable to ascertain the cause.

At about this point, after the writer had experienced itching and burning sensations on the forearms for the third time, he began to suspect where the trouble lay and thus proceeded to experiment with tussock-moth cocoons. Three different people submitted to a test, that of rubbing the cocoons against the forearm. In each case an itching and burning sensation began within three minutes after the cocoon was applied. This was followed shortly by a distinct swelling which continued to be painful for several hours. After about 24 hours the swelling would subside but red spots were visible on the third day. Two people who submitted to having a small spot on the forearm rubbed by a cocoon, suffered to such an extent that sleep for the following night was considerably disturbed. In all, seven people were tested for the toxic effect from the cocoons and larvae, and in each case swelling and painful results followed. It did not seem necessary to ask a larger number of people to submit to the test in order to prove the poisonous

* Contribution from the Entomological Department of the University of Minnesota.

nature of certain hairs on mature larvae of *Hemerocampa leucostigma* and their cocoons.

A review of the literature in search of information regarding the poisonous nature of the tussock-moth is interesting. Perhaps the first and most incriminating evidence against the tussock-moth is reported by Howard (1896) in which he says: "The barbed hairs just mentioned may occasionally produce considerable irritation of the skin of people upon whom the caterpillars may have crawled or dropped from the trees. The hairs from the different portions of the body of the full-grown caterpillar are illustrated, greatly enlarged, in figure 88, and it is the shorter hairs from the sides which probably cause the irritation. They are very small, fall out readily, and when a caterpillar crawls over the skin of an individual who is warm and perspiring, these very sharply barbed hairs produce an irritation which in some individuals has been the cause of much discomfort, creating more or less inflammation and swelling."

Göldi (1913) reproduces Howard's figures and cites the tussock-moth as the most poisonous of American caterpillars. Apparently, little is said by American authors to support this view. Riley and Johannsen (1915) seem to reflect the general opinion of entomologists in this country when they state: "Göldi (1913) . . . has through some curious misunderstanding featured the larva of *Hemerocampa leucostigma* as the most important of the poisonous caterpillars of this country." Perhaps the real truth regarding the tussock-moth lies along some middle ground.

Kephart (1914) has shown that the brown-tail moth (*Euproctis chrysorrhoea* Linn.) derives its poison from glands situated at the base of certain hairs. The writer believes that similar structures will be found producing poison for the tussock-moth. An investigation of this subject is now under way at the University of Minnesota, and we hope in due course of time that the exact nature of the poison hairs of *Hemerocampa leucostigma* will be made known.

The writer found that the poisonous hairs of the tussock-moth caterpillar are located in the prominent tufts on the dorsal surface. When these tufts were rubbed against the skin, groups of barbed hairs could be seen under the lens, apparently hooked in the pores of the skin. These hairs would break easily and could scarcely be removed by aid of forceps. The flimsy cocoons which this species makes are interwoven with the hairs from the body of the caterpillar, thus the poison hairs still serve to protect the pupa against certain forms of molestation.

There is some indication that the poison glands may not be fully developed until the larva is nearly full-grown. If this were the case it might account for two things: (1) The writer discovered the poisonous nature of the tussock-moth caterpillars at a time when nearly

all were mature and starting to spin cocoons. Younger stages of the larva were difficult to find. Only one half-grown larva was tested for toxic effects, and this one failed to react as did seven or eight mature caterpillars. (2) If the poison is developed only in the late instars of larval life, such might account for the times when people have handled tussock-moth caterpillars without noting any poisonous effects.

Fabre in his "Life of the Caterpillars" gives the results of several experiments with European caterpillars from which he draws the conclusion that it is the excreted products which accumulate on the hairs, or when the same toxin is obtained in concentrated solution from the excrement, which gives a distinctly poisonous reaction when applied to human skin. Fabre evidently believed that all caterpillars which are poisonous, owe that fact to the virus collected on the hairs by rubbing against excrement. He did nothing, however, on the morphology of hair structures which would exclude the possibility that the poison may, even in the species with which he worked, be derived from glands at the base of certain hairs.

While testing old remedies to obtain relief from the poison of the processionary caterpillar, Fabre found that the common garden purslane proved to be a very efficacious palliative. Accordingly, the writer tried purslane (*Portulaca oleracea* L.) as a palliative for the poison of the tussock-moth larva, mashing the leaves into a mucilaginous pulp and applying this to the swollen spots. The relief obtained was surprising. Purslane was then tried as an experiment on three different people who had swellings caused by being rubbed by a tussock-moth cocoon. All were agreed that the relief was very pronounced.

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A STUDY OF TRYPANOSOMA AMERICANUM

By R. W. Glaser

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

On using some cattle blood for other work, the writer encountered the very large haemoflagellate first described by Crawley in 1909 under the name of *Trypanosoma americanum*. Later in 1912, Crawley more fully described the morphological, cytological and cultural characters of this interesting form. Since the appearance of Crawley's publications much work has been done on Herpetomonas, Crithidia, Leishmania, Trypanosoma, and other genera. By the application of the methods of Novy, the latter investigator and his students, and workers like Laveran, Franchini, Prowazek, Nöller, Wenyon, Kofoid, Fantham, Porter, McCulloch and others, paved the way for a scientific understanding of the true relationships of forms which ontogenetically and phylogenetically had seemed rather doubtfully interested in one another.

It therefore seemed to the writer that a restudy of Crawley's large species might contribute some facts not so easily obtained from smaller forms. *Trypanosoma americanum* is not only a very large species, but it is easily obtained and grown. It occurs around Washington, as Crawley found, in the blood of 74 per cent. of the cattle. The present writer's results on the incidence of infection in New Jersey are not comparable with the total percentage given by Crawley, for the reason that the former's work was begun after the first of October, 1921, and Crawley showed that: "By October the trypanosomes have become much less abundant in the blood, and an animal negative in that month might readily have been positive in July or August." The other purpose of the present investigation was to disclose, if possible, a few facts bearing on the parasitic or pathogenic possibilities of *T. americanum* and the manner of transmission from cow to cow.

During the months of October, November and December, cow blood was drawn under sterile conditions from the jugular vein at intervals. Following the cultural directions of Crawley, tubes were prepared containing 6 cc. of the defibrinated cow blood to be tested, above which was placed 6 cc. of sterile bouillon. The tubes were incubated at room temperature and the upper layer of erythrocytes examined for trypanosomes at the end of 3 or 4 days.

The accompanying table shows the number of cases that proved positive for trypanosomes.

Cultural Requirements.—As Crawley showed, the trypanosomes are so rare numerically in cattle that the preparation of cultures offers the only safe method for determining their presence. However, the present writer centrifuged 50 cc. of fresh defibrinated blood from cow 584. The blood from this cow had previously yielded cultures of trypanosomes. After centrifuging, the bottom layer was examined in a fresh state on several slides with a low power objective. After a long search one *T. americanum* was found. This specimen was very large and morphologically similar to the forms found in early cultures, 12 to 48 hours. The undulating membrane was short and ended anterior to the center of the body. No other specimens were found although others were doubtless present for the same blood from which the samples for centrifuging were removed yielded cultures later.

TABLE SHOWING INCIDENCE OF INFECTION IN COWS AND YEARLING HEIFERS

Date 1921	Cow	Results	Date 1921	Cow	Results
Oct. 15	584	+ +	Nov. 8	Calf 938	— —
Oct. 15	600	— —	Nov 10	735	— —
Oct. 15	601	+ +	Nov. 10	722	— —
Oct. 25	584	+ +	Nov. 10	757	— —
Oct. 25	600	— —	Nov. 10	896	— —
Oct. 25	601	+ +	Nov. 10	758	— —
Nov. 8	3750	— —	Dec. 12	1096	— —
Nov. 8	843	+ +	Dec. 12	1092	— —
Nov. 8	45	— —	Dec. 12	1085	— —
Nov. 8	3877	— —	Dec. 12	1091	— —
Nov. 8	859	— —	Dec. 12	1075	— —
Nov. 8	3881A	+ +	Dec. 12	1064	— —
Nov. 8	878	+ +	Dec. 12	1063	— —
Nov. 8	8858	— —	Dec. 12	1077	— —

Six cc. defibrinated blood were used for each culture. Two cultures were prepared from each sample of blood.

The cultural requirements and characters of *T. americanum* in liquid media were fully described by Crawley. The writer may add that he had just as good success with horse blood as when cow blood was used. However, after 5 or 6 days the trypanosomes begin to hemolyze the top layer of erythrocytes and it becomes difficult to see the colonies at the junction of the red cells and bouillon. This phenomenon does not appear to occur so rapidly with the cow blood cultures.* They remain clear for quite a long time and consequently one can see well enough to fish out individual colonies with a fine pipette.

The N. N. N. solid medium prepared either with cow or horse blood also proved highly satisfactory. As will be shown later, forms are

* It is important to use fresh blood for culture media.

obtained on this medium not found in liquid cultures. Moreover, the trypanosomes grow very rapidly on the N. N. N. medium during the first 4 or 5 days, and consequently it is a favorable source for the demonstration of dividing individuals. In applying it, however, care must be taken to keep the surface moist by adding sterile horse or cow serum every few days or by placing the petri dishes in a humid atmosphere. Colonies do not develop on the N. N. N. medium; the growth is diffuse. In order to obtain the best results all the cultures should be kept at room temperature; the higher incubator temperatures, 35 to 37 C. inhibit growth and produce degeneration and death in 3 to 5 days.

Cytology of the Stages Found in Cultures

In the study of this phase of the subject preparations were made from the various cultures at intervals. Some of these preparations were studied in the fresh condition or with intravital dyes. Others were fixed and stained. Crawley dried his films, staining them later with Wright's stain. The writer repeated this procedure, but abandoned it in favor of the "wet method," for the reason that much better and more beautiful preparations resulted. As a general routine, the films were drawn out very thinly and then immersed, while still wet, for 15 minutes in half and half absolute alcohol-ether mixture. They were then stained by Novy's modification of the Romanowsky method. This gave by far the best results, although certain cytologic structures could be better elucidated by using Schaudinn's fixative and Heidenhain's iron hematoxylin. Mallory's chloride of iron hematoxylin method was also used to advantage in a few cases.

The cultures are best studied 3 days after incubation at room temperature. At this time a sufficient number of trypanosomes have multiplied so that one has little difficulty in finding them. In 3 days the bodies of *T. americanum* averaged 17 to 18μ long. The free part of the flagellum was usually as long as the body or slightly longer. Often enormous forms were encountered. Some of these measured 25μ long or longer, exclusive of the flagellum.

In trypanosomes taken from liquid cow blood cultures 3 days old (Figs. 1, 2, 3, and 4), the body is long, more or less cylindrical and rather flexible. The flagellum on reaching the anterior part of the body becomes an undulating membrane which follows the length of the body laterally for about one-third or less of the distance. It then loses itself in the body of the trypanosome ending (Figs. 1 and 2) near a dark staining granule, the parabasal body. No blepharoplast or thickening of the end of the thread itself can be detected. The nucleus, when demonstrated, is located centrally (Fig. 1) or anterior to the central (Fig. 2). A karyosome is usually clearly differentiated. The end of the undulating membrane may be some distance from the nucleus (Fig. 1)

or the two may lie closer together (Fig. 2). In many specimens large and deeply staining granules are distributed throughout the finely granulated cytoplasm. When these granules lie in close proximity to the end of the undulating membrane, it is difficult to determine which one constitutes the parabasal body. However, specimens such as the one represented in figure 2 are frequently found. In these cases only one large deeply staining body is found in the cytoplasm; it is in close proximity to the end of the thread and is consequently interpreted as the parabasal body.* Cases were found (Figs. 3 and 4) in which no nucleus was visible (unless one interprets the large deeply staining granules near the center of the body as such). These cases are difficult to interpret because they were present on slides containing other trypanosomes with well fixed and differentiated nuclei.

In specimens found in 8 day liquid cow blood cultures (Figs. 5 to 9), nearly all individuals have lost the undulating membrane. Many of the organisms have approached the typical herpetomonad type. Figure 5 represents such a case; no undulating membrane, rigid body with the nucleus in an extreme anterior position. Figures 6 and 7 represent bulbous forms with flagella; figures 8 and 9 more extreme types with that organ lacking. In figure 9 nuclear division seems to have occurred. (The writer doubts whether this is a futile attempt at endogenous budding.) Figure 10 represents one of several organisms found in an 8 day horse blood culture showing that some of the bulbous forms are still capable of reproduction.

Forms found in 14 day old cow blood cultures (Figs. 11 to 19) show bulbous types with flagella, and round or oblong ones without that organ and not simulating flagellates in the least. The discovery of such forms as those shown in figures 15 to 18 aroused the writer's suspicions that he may be confronted with the result of a kind of endogenous budding similar to that found by McCulloch (1919) in *Crithidia cmryophthalmi.* A diligent hunt revealed other stages such as that represented by figure 20 from 14 day old cow blood culture, and figure 21, found in a 20 day old cow blood culture. These stages are very rare and the writer has studied his cultures enough to feel sure that they do not develop, but abort. These structures seem to hint at either a phylogenetic recapitulation, or at certain latent developmental possibilities, and if any stages in the invertebrate host exist and were known a further expression of opinion might be possible.

In 20 day old cow blood cultures many degenerating forms appear. After 20 days the cultures gradually die out although a few forms may persist for a much longer time. Figures 22 to 23 represent two unusual

* The nomenclature of Kofoid, Swezy and McCulloch is followed in describing the extranuclear organelles.

forms seen in a 20 day old cow blood culture. In figure 22 a short undulating membrane exists ending definitely at a well defined parabasal body which is here situated alongside of the nucleus. In figure 23 the nuclear system seems to have migrated posteriorly. On the N. N. N. medium with either cow or horse blood development is much more rapid and consequently degeneration occurs much sooner. The life of the trypanosomes on the solid medium is not nearly so long as is the case when it is grown in the blood bouillon mixture.

On 4 day old cultures grown on the N. N. N. plates such forms as those on Plate III appear. Figures 24 and 25 show types similar to those found in early liquid cultures with the exception that the organisms are smaller and the body more attenuated. Figures 26 and 27 represent respectively a large and a small herpetomonad type. Due to the rapid multiplication on the N. N. N. medium one readily obtains many division stages. Figure 28 shows nuclear and cell division, but the parabasal body is still intact. Figure 29 shows another form undergoing nuclear division. Figure 30 demonstrates nuclear, parabasal body, and cell division. Figure 31 represents another form of division superficially simulating constriction. There is no evidence for any other common method of division excepting longitudinal, therefore what happened here was partial longitudinal division accompanied by a movement of the two halves in opposite directions before final separation occurred. Figure 32 shows what appears like an abnormality, cell division without any apparent preceding nuclear division. Figure 33 represents a group of trypanosomes including actively growing and dividing forms, and senile and degenerating individuals. Figures 34 and 35 represent still other conditions encountered.

An examination of the N. N. N. plates in 7 days shows many curious shapes and forms that can only be interpreted as degeneration stages. In 9 days the degeneration stages are well accentuated and the protozoa have a tendency to become very large and amoeboid as shown in fixed and stained preparations. No amoeboid activity can, however, be determined in fresh films examined at room temperature or on the warm stage. Plate IV shows a series of degeneration forms. The appearance is strikingly like that of an amoeba. The cells are large with reticulated cytoplasm containing often many deeply staining granules. Figure 36 still possesses its flagellum, a telltale brand of the organism's flagellate origin. The nucleus has a well defined karyosome and chromatin granules within the clear nuclear substance. Figures 37 and 38 also show large nuclei filled with chromatic material, and figure 39 shows a karyosome as well. Figure 40 shows a long attenuated form. The cells although senile are still in some ways physiologically functional. The nuclei, as has been shown, are normal and the organisms are still at times capable of reproduction (Fig. 41). After about 12

to 14 days, however, the protoplasm of the organisms becomes coarsely granular, Brownian movement is seen within, and complete disintegration soon follows.

Specificity

Since *Trypanosoma americanum* occurs as a parasite in American cattle in which it produces no visible pathologic conditions, little hope was experienced that the inoculation of laboratory animals would throw any light on the nature of this parasitism. Nevertheless, two guinea pigs, one rabbit, six white and two wild mice were inoculated with young, 3 to 4 day old, cultures. Intraperitoneal inoculation was practiced on the guinea pigs, intravenous on the rabbit and subcutaneous, intraperitoneal and intravenous inoculation on the mice. Blood smears were made from time to time from all animals with negative results. Temperatures and weights demonstrated nothing.

One guinea pig was autopsied in 3 weeks. All the organs were carefully examined and cultures made with negative results. The mice, which were given from ¼ to 2 cc. of the cultures, dependent on the manner of inoculation, were all autopsied at intervals from 4 days to 2 months. Smears and cultures were made from the peripheral and heart blood, the spleen, liver, kidneys, and bone marrow, but no trypanosomes or stages of trypanosomes were found in smears or recovered in cultures. These results, taken in conjunction with the observations already published on cattle by Crawley, seem to indicate that *T. americanum* is specific to cattle.

It appeared to the writer that an invertebrate host, viz., some insect, might act as the transmitter of *T. americanum*. The insect fauna occurring around the cattle that harbored trypanosomes was carefully studied and only three forms appeared worthy of consideration, namely, *Stomoxys calcitrans, Haematobia serrata,* and *Musca domestica*. It may seem unnecessary to include the latter species, but it has been shown that the house fly will often "follow up" the bites of the true blood-sucking forms and under these conditions will engorge, so that consequently it may be a source of infection and transmission. Moreover, Darling (1912) showed that the house fly is capable of transmitting *T. hippicum* to healthy mules.

Collections of the three flies were made from the bodies and stables of animals known to harbor *T. americanum*. These flies were carefully dissected and the entire alimentary canal including the crop, salivary glands, and Malpighian tubes was examined. Ninety-two house flies, 147 stable flies and 85 horn flies were thus investigated, but no trypanosomes were found. *Herpetomonas muscae-domesticae* was frequently encountered in house flies, but no other protozoa were seen in the two blood suckers. These negative results should not discourage the point

of view, still held, that one of these three flies will prove to be responsible for the transmission of *T. americanum.*

In order to determine whether *T. americanum* could survive in flies, 20 Stomoxys and 10 Haematobias were fed with a vigorous liquid culture of this flagellate. Two Stomoxys and two Haematobias were dissected immediately after engorgement and trypanosomes were found in abundance, showing that the flies actually ingested the organisms. Four Stomoxys and four Haematobias were dissected in 24 hours. The flagellates were found in the intestines of all, but had experienced a considerable modification. Many herpetomonad types were seen; rigid, attenuated with no undulating membrane. In 48 hours four more Stomoxys, and the remaining Haematobias were examined. In two cases what appeared like a few degenerating flagellates were seen. All of the remaining Stomoxys were dissected in 72 hours and nothing was found. The flies during this time were well cared for and supplied twice daily with defibrinated horse blood which they ate. This experiment seems to show that Stomoxys and Haematobia might act as the transmitters of *T. americanum,* but that the transfer from host to host must occur within 48 hours. The natural mode of transmission remains problematical, however, until *T. americanum* has actually been found in the intestine or some other organ of a fly caught wild.

Modifying Influence of the Environment, and Taxonomic Position

Recently Nöller (1920) has cast doubt on the validity of the genus Crithidia and places this genus in synonymy with Trypanosoma. This investigator worked with crithidia-like organisms morphologically very similar to *Trypanosoma americanum.* Nöller showed that trypanosomes could be changed into Crithidia and vice versa. He subjected the sheep trypanosome, growing on sheep blood agar, to different temperatures. At 30 C. the organism grew as a Crithidium with a short undulating membrane. At 37 C., in a few days, the body became snake-like, the rigid hind end became flexible and intermediate stages between Crithidia and true blood trypanosomes began to appear.

In the case of the trypanosomes of birds the same phenomenon occurred. At room temperature the organisms grew as Crithidia, but when subjected to 37 C. for 48 to 72 hours true trypanosomes appeared, although multiplication ceased at that temperature. If the plates were returned to room temperature rapid multiplication resumed in 12 to 48 hours, and the organisms changed again into Crithidia. Nöller also states that Trautmann succeeded with difficulty in converting the crithidial phases of *T. theileri* (an organism parasitic in European cattle) into trypanosomes by subjecting them to a temperature of 37 C. for from 5 to 8 days. From these experiments Nöller concludes that the genus Crithidia is a synonym for Trypanosoma.

Since *T. americanum* is morphologically really a Crithidia the writer repeated Nöller's experiments with this form. The experiments are given in detail below.

1. *Trypanosoma americanum* was grown on cow blood agar (2 plates) at room temperature 3 days. At this time an examination showed many active Crithidia with anterior nuclear complex and short undulating membranes. The plates were then placed in incubator at 37 C. and examined in 2 days. Multiplicative energy had ceased and among Crithidia many deformed individuals were found, such as bulbous and round forms. No trypanosomes were seen. In 4 days the organisms were found dead and disintegrating with the exception of a few greatly deformed individuals. This experiment was repeated at 35 C. with a similar result. No trypanosome types ensued.

2. *Trypanosoma americanum* was grown in liquid cow blood bouillon mixture (4 tubes) at room temperature for 3 days. At this time an examination showed active Crithidia with the anterior nuclear complex and short undulating membranes. The tubes were then placed in the incubator at 37 C. One day later the cultures were examined. Multiplication was inhibited. The organisms were crithidia-like with anterior nuclear system and short undulating membranes. The cultures were again examined in 2, 3, 4, and 5 days, but no conversion into true trypanosomes occurred. Deformed and degenerating individuals began to appear and in 6 to 7 days the cultures had "died out."

This experiment was repeated at 35 C. with a similar result. No trypanosome types ensued.

It might be well to state that all of these cultures were not only studied in the fresh state, but all results were checked with fixed stained preparations.

The above experiments prove that by the method employed the crithidia-like *T. americanum* was not converted into a true trypanosome.

The writer has never seen *T. americanum* approach the trypanosome type under any conditions. In cultures, as was shown above, herpetomonad types are common. These types were also observed, as was shown with the fly experiment, if the crithidial forms are introduced into the invertebrate intestine. Moreover, the examination of very early cultures made from cattle blood always revealed the crithidial type as both Crawley and the writer found. Lastly, the experiment with the freshly drawn and centrifuged blood mentioned in the beginning is also proof of the fact that the crithidia-like phases of *T. americanum* obtained in cultures resemble the forms as they occur within cattle.

It would seem then that the organism under discussion is a Crithidia and not a trypanosome. For morphological reasons this would seem to be the case. In the trypanosomes, the extranuclear organelles are at one time in the natural life cycle, or on some medium, or at some temperature according to Nöller, located posteriorly to the nucleus. Such forms have a well developed undulating membrane. These characters are common to trypanosomes found in the blood. *T. americanum* occurs naturally in the blood, yet the blood forms do not possess these characters nor can they be experimentally induced to assume them. In common with most Crithidia the nucleus is usually

centrally located or anterior to the center. The extranuclear complex is located anterior to the nucleus and a short undulating membrane exists.

In spite of the crithidial nature of *T. americanum,* the writer feels it best not to include this flagellate in the genus Crithidia. The true Crithidia are forms inhabiting the alimentary tract of invertebrates, often in the intestines of plant-feeding insects. Such flagellates have never had occasion to come in contact with vertebrate blood and it would be impossible to predict what might happen to them should this occur. Since the organism under discussion resembles a Crithidium morphologically, but naturally lives in the blood of cattle, it seems best to regard it as an intermediate evolutionary stage between the true crithidians and true trypanosomes. For this reason, and on account of the fact that Crawley's organism has remained in the genus Trypanosoma so long, the writer prefers not to alter its taxonomic status.

In conclusion I wish to state that I am indebted to Dr. Ralph B. Little of this department for collecting the samples of blood.

SUMMARY

Trypanosoma americanum was successfully grown in horse blood medium and on the N. N. N. medium, as well as in cow blood medium. Development in the culture media was traced, and the cytological details of the various stages described.

T. americanum is specific to cattle. In freshly drawn blood and in very early cultures *T. americanum* resembles the majority of the forms found in 3 and 4 day old cultures.

Morphological and experimental data are presented to show that *T. americanum* is structurally a Crithidium. Prolonged culture and environmental alterations have a tendency to produce herpetomonad types but never trypanosome types.

Reasons are presented in support of the view that *T. americanum* is an intermediate evolutionary stage between true Crithidia and true trypanosomes. The name *Trypanosoma americanum* is retained.

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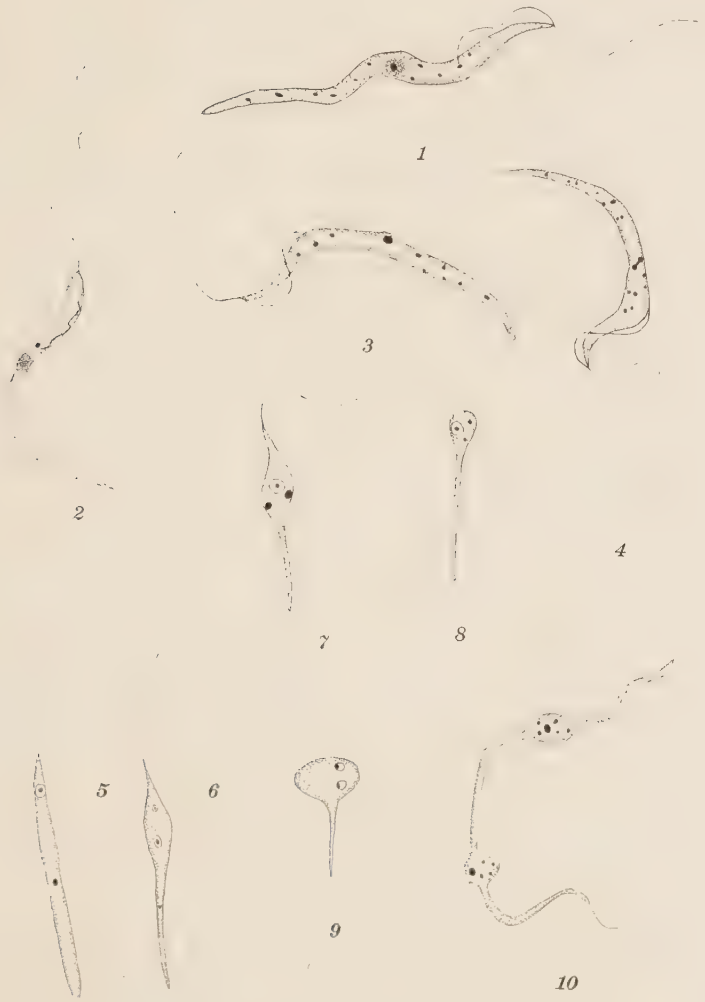

PLATE XIV

All figures represent *Trypanosoma americanum*. × 813.

Figs. 1-4.—Trypanosomes from 3 day liquid cow blood cultures. Figs. 5-9.—Specimens from 8 day liquid cow blood cultures. Fig. 10.—Specimen from 8 day liquid horse blood culture.

PLATE XV

Figs. 11-20.—Trypanosomes from 14 day liquid cow blood cultures. Figs. 21-23.—Specimens from 20 day liquid cow blood cultures.

PLATE XVI

Figs. 24-35.—Trypanosomes from 4 day N. N. N. cultures.

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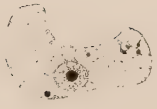

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PLATE XVII

Figs. 36-41.—Trypanosomes from 9 day N. N. N. cultures.

FLUKE INFECTIONS AND THE DESTRUCTION OF THE INTERMEDIATE HOST*

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Two articles have recently appeared which demand more than passing notice by the biologist and fish culturist. An editorial in the Journal of the American Medical Association (1921) calls attention to methods proposed by Chandler (1920) for the purpose of exterminating certain dangerous flukes by eradicating their intermediate host (namely, mollusks), and strongly recommends the prophylaxis suggested. In view of the wide circulation of this medical journal, and of the harmful results that would result from the general application of the remedies suggested, it seems necessary to draw attention to the general relationships of fresh water animals and the consequences of interfering in too large a degree with the normal activities of a biota.

It is realized that in certain localities fluke infection does a vast amount of harm to both man and domestic animals, and methods for the elimination of this disease are to be welcomed by all people interested in the welfare of the human race. The remedy suggested by Chandler, however, is so far-reaching in its results, in directions probably not considered by the author, that it calls for comment and vigorous protest by those interested in the conservation of our aquatic resources.

Two classes of water bodies are considered by Chandler; one the small swale or pool, filled with water during the wet season (winter and spring of the north), and dry or but partly filled with water in the dry season (summer and fall of the north). As these pools are more or less temporary and contain no life of great biologic or economic importance, the eradication of mollusks and other forms would be clearly desirable for the control of the fluke, as well as for the destruction of the malarial-bearing mosquito larvae, especially in endemic areas. But many of the species of mollusks harboring intermediate stages of flukes inhabit running streams, lakes, and large ponds, and in these cases to apply the remedy suggested would not only affect the snails in question, but also other associated animals, including fish.

In his paper Chandler remarks that "Experiments by the writer, as well as by others, show that copper, one part per million, is not injurious, *at least within* 48 *hours*, to annelids, crustaceans, or aquatic

* Contribution from the Museum of Natural History, University of Illinois, No. 24.

insect larvae. Of vertabrate animals, *fish are highly susceptible, various species being affected by* 1 *part of copper sulphate in from* 500,000 *to* 10,000,000 *parts of water.* . . . It is evident, therefore, that copper salts in high dilution have a selective effect on various organisms, being particularly destructive to single-celled organisms, certain molluscs, *and fishes"* (italics are the writer's). The effect of copper salts on the fish fauna of a stream, if this method of eradication were to be carried out on a large scale, may well be imagined.

During the past ten years or more, attention has been largely directed to the question of river and stream pollution by sewage and chemicals, and biologists and sanitary engineers have written extensively on the subject (see Ward, Forbes and Richardson, Shelford, Baker). Sewage, highly concentrated, has been shown to be very injurious to mollusks and other animals, and to eradicate them in a few years (Baker, 1920). In the Allegheny River in Pennsylvania, mine water containing acids has effectively destroyed all forms of animal and vegetable life in sections of the river (Ortmann, 1909). The use of copper salts in sufficient strength to eradicate all possible hosts of flukes in streams and rivers would in a short time destroy fish as well as the food upon which they subsist.

The general interrelationships of the inhabitants of water bodies are not always well understood by either biologists or medical practioners. A noted student of fresh water biology has aptly said that a body of water is a microcosm in which all of the activities of life go on uninterrupted, quite independent of the land, which, for practical purposes, might be eliminated without seriously affecting the water population. All of the inhabitants of these microcosms are interdependent; a destruction of one groups affects all. Animal life is dependent, in the final analysis, on vegetable life, the great majority of invertebrate animals being herbivorous in food habits (over 99 per cent. are herbivorous or detritus eaters; see Baker, 1918:205). Copper salts are especially toxic to all forms of algae (one of the commonest foods of invertebrate animals) and the destruction of mollusks as hosts of flukes would also destroy the algae on which many groups of animals feed—amphipods, chironomid larvae, obligochaete worms, ephemerid nymphs, etc. (Baker, 1918:205). This in turn would deprive the carnivorous forms of their food and the result would be the complete elimination of the fauna and flora from the body of water treated.

Many of the molluscan hosts live in the smaller streams, although all genera of hosts live also in the largest lakes and rivers, and the question may be raised as to whether the life of these small streams is of any consequence, economically. Studies of fish culturists and biologists have shown that these small streams are of the greatest value,

providing favorable spawning places for adult fish and feeding places for the young which later migrate to the larger bodies of water. The smaller species of fish which are not of any direct economic importance are of indirect value in furnishing food for the larger carnivorous food and game fishes, and their destruction would result in great harm to the fresh water fisheries of our country. Copper salts would also be inimical to the river mussels (from which pearl buttons are made), both in the adult stage and, especially, in the glochidial or embryonic stage, during which time they are completely dependent on fish for development (see Coker, et al., 1921).

In conclusion it may be pointed out that the suggested control of such snails as are intermediate hosts of certain species of flukes inimical to man and the domestic animals, while applicable to small bodies of water not connected directly with running streams or large lakes, is absolutely impracticable for running streams and larger bodies of water. To successfully eradicate all possible intermediate hosts of flukes would necessitate the pollution of water bodies to such an extent that most forms of life would be ultimately killed; in comparison with this toxic agent, when used effectively, our present stream pollution by sewage would sink into insignificance.

Chandler's experiments are of the highest interest and value and their practical application in special places, especially in endemic areas, will doubtless be effective, and desirable. It must be pointed out, however, that the methods suggested could not be used on a large scale without seriously affecting all aquatic resources and several important industries dependent upon fresh water life for raw material.

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A SOURCE FOR MATERIAL OF PROTOZOAN AND OTHER PARASITES

Some years ago the writer sent to the Zoological Station at Naples for intestines of the marine fish *Box boops,* preserved by several different methods. The material preserved in alcohol proved to have the Opalinidae of the intestines in a good state of preservation. This suggested the possibility that museum specimens of Anura might have their Opalinidae sufficiently well preserved for taxonomic study. This proved to be the case. Over 1100 good infections of Opalinids were found in the Anuran material of the United States National Museum. In many cases the material was good enough for study of nuclear phenomena which were used in specific distinctions, the number of macrochromosomes being a very usable diagnostic character. Animals that had been from 40 to 80 years in their hosts in alcohol upon the Museum shelves yielded good Opalinid material. Other Ciliates also were well preserved, as were also Trematodes and, of course, Nematodes.

Specimens of Anura are usually preserved in alcohol of not more than 70% strength. When very strong alcohol was used the Opalinids were well preserved, but the tissues of the host were so hard as to make it difficult to obtain the parasites without injury to the hosts. A number of frogs were opened which had evidently lain long in very weak alcohol, their tissues being so flabby as to tear readily, but even in such flabby specimens there were found Opalinids well enough preserved for taxonomic study. On the other hand, Anura preserved in formalin seldom show any Opalinids and these, when found, are difficult to stain and are unsatisfactory for study. The flukes also are in poor condition. Generally the intestinal lining itself is degenerate and broken down, being scattered in flakes through the lumen of the intestine.

Rectal parasites can be removed from preserved specimens of Anura without doing appreciable injury to these specimens. A curved cut on the ventral surface, a little to the right of the mid line, enables one to lift a flap of the abdominal wall and expose the rectum. A slit through the rectum, long enough to admit a very narrow spatula, allows collection of the rectal contents with its Opalinids and other parasites. All this really does no harm to the specimen as a museum specimen. Indeed good collectors are accustomed to slit the abdomen in Anura before placing them in the preserving fluid.

Zoological explorers and others preserving material for general study should have in mind not only the animals themselves, but their parasites as well. Consideration for the protozoan parasites would lead to preservation of Anura in alcohol and not in formalin.

If organisms so delicate as Ciliates are found well preserved in museum specimens of Anura, there are doubtless many other parasites to be found in similarly preserved material of other groups. There is here a mine of abundant material for parasitological studies. Not many museums would be so generous as the United States National Museum in allowing search of all their preserved specimens in a group for parasite material, but most working museums would have much accessory material which they would be glad to have so used, and around most laboratories are many jars of unused material in which must be parasites well enough preserved for taxonomic study.

The Orchard Laboratory, Oberlin, Ohio Maynard M. Metcalf

NEW HUMAN PARASITES

Pirobodo intestinalis Sangiorgi 1922.—G. Sangiorgi (Pathologica, 13: 69-71, figs. 1-5) observed a flagellate with two flagella in the feces of soldiers suffering from diarrhoea during the anti-dysentery campaign in 1918 in Albania. When young the flagellate is elongated, measuring 8 to 9.6μ long by 3.2 to 4.8μ broad; in more grown stages they measure 12.8 to 16.6μ long by 9.6 to 14.4μ broad. The body is pyriform with broadly rounded posterior tip. The cytoplasm is uniformly granulated in the young stage, but becomes highly and peculiarly vacuolated in the posterior half of the body as the animal grows. The nucleus is a rounded structure. Two flagella similar in length have their point of insertion at the attenuated anterior tip. The cysts measure 9.6μ in diameter. Division probably takes place by longitudinal fission. Sangiorgi establishes a new subgenus Pirobodo for the flagellate under the genus Bodo.

Spirochaeta perforans Cavalié and Mandoul, 1921.—In 23 cases of expulsive alveolo-dental polyarthritis with purulent or non-purulent exudate, a species of spirochete was found constantly present. This organism was unmixed with other micro-organisms in the peripheral portions of the lesions bordering on the healthy tissues. It measures 10 to 13 microns long by about 2 microns in thickness. The ends are blunt without apparent flagella. The number of spirals in some instances ranges between three to five, in others between seven to nine, these two forms at first suggesting two species, but finally considered by the authors to be two varieties of the same species. (C. R. Soc. de Biol., 85: 1068-1069).

BOOK REVIEW

THE INTESTINAL PROTOZOA OF MAN. By Clifford Dobell and F. W. O'Connor. William Wood & Co., New York, 1921. 221 pp., 8 pl.

This book represents primarily the results of observations upon the intestinal protozoan parasites of man, found and studied by the authors in England, during and since the world war, together with brief reviews and criticisms of the papers by other investgators upon these organisms.

The text is divided into nine chapters. A general consideration of the Subkingdom Protozoa and its four Phyla with other allied matters and the diagnosis of intestinal protozoal infections in which is given an introduction to the technique of studying the parasitic Protozoa, form two chapters. Descriptions and keys of the known species of amoebae (4 genera and 5 species), flagellates (5 genera and 5 species), coccidia (2 genera and 4 species), and ciliates (2 genera and 3 species) of man, are given in order in separate chapters which also deal with the pathology and symptomatology of the respective infections. The outstanding feature of this book is the earnest cooperation of a careful worker in zoology and of an experienced medical man; and this is manifested in the careful combination of medical and zoological statements in all topics.

The book is wide and fairly complete in its scope as far as present knowledge is concerned; it is clearly and briefly written in an attractive style and may, on the whole, answer well the purposes aimed at by the authors. Yet the general information given in the first chapter seems to be too brief, for some readers may easily acquire a misconception of some of the general characteristics of Protozoa (for instance, those of Sporozoa). Under the limitations of space here a clear fundamental knowledge of the general subject of Protozoa can be had only by referring to other comprehensive works.

In the chapters dealing with amoebae and amoebiasis which are in the main condensations of one of Dobell's former papers (*Amoebae living in Man*, 1919; reviewed in THE JOURNAL, 6:202), Dobell seems to be positive about the nature of the so-called "iodine cysts." The views expressed by Kofoid and his colleagues (1919), Brug (1921) and Nöller (1921), however, seem to demand further careful investigation of this feature.

Although Dobell concluded his manuscript in April, 1921 (p. ix) and has quoted three of his own papers in that year, he failed to mention or discuss two species of parasitic amoeba recorded in 1920: *Entamoeba paradysenteriae* Chatterjee 1920, reviewed in THE JOURNAL, 8:48) and *E. macrohyalina* Tobaldi 1920 (reviewed in THE JOURNAL, 7:102). It is further regretful that the study by Kofoid and Swezy on an amoeba of a new genus: *Councilmania lafleuri* 1921 (reviewed in THE JOURNAL, 8:48) from North America was published a few months after this date and hence could not be included in the present work.

Of the flagellates Dobell considers that the cysts of *Trichomonas hominis* have not yet been found by anyone. One must agree with him in maintaining that the cysts observed by Lynch (THE JOURNAL, 3:28-33) were really those of *Chilomastix mesnili*, but can hardly find adequate reason to join him in interpreting the cysts described by Boyd (THE JOURNAL, 5:132-136) as "merely rounded and degenerating individuals." As to the cultivation of this flagellate *in vitro*, Dobell concludes that "at present, however, it is not possible to cultivate this organism with certainty in any medium," since "all the attempts which we ourselves have made have been failures." American students of Protozoology are nevertheless well acquainted with the positive results obtained by several authors in artificial cultivation of these flagellates. Most recently Hogue (1921) has succeeded in cultivating this organism in a new medium. The statement just quoted takes the form of an unjust criticism of the studies by Kofoid and Swezy on *Chilomastix mesnili*. After quoting their descriptions of

the structures of this flagellate, Dobell writes that "even if their account is correct—which we do not believe—it appears to be physically impossible to prove the existence of so many structures in so small a cyst (about 8μ in diameter), for they figure systems of points and lines whose actual existence could hardly be demonstrated by the finest optical apparatus." Furthermore, concerning nuclear division in the cyst of the flagellate as described by Kofoid and Swezy, Dobell states that "if nuclear division does occur within the cyst, it must be excessively rare" and that he is "completely at a loss to account even for the single nuclear division which they describe," since he never saw a single cyst containing more than one nucleus, though having examined "thousands upon thousands of cysts in human feces." In this instance Dobell seems to have overrun the limits of appropriate criticism, in view of the fact that he does not state he has ever examined the preparations of the above mentioned American investigators, and further in view of the statement to the effect that, since he has not seen a cyst with more than one nucleus, the nuclear division described by Kofoid and Swezy in the cyst of *Chilomastix mesnili* must be a false interpretation of the "optical image."

The synonym of the seventeen species of Protozoa is given thorough consideration, and their geographical distribution is generally though briefly discussed. Yet, if the names of the localities where the Protozoa had hitherto been observed were even merely listed, readers would have been greatly benefited. The treatment of intestinal protozoal infections is dealt with in desirable detail and will give readers a fairly clear view of the subject without referring to the original papers.

The last chapter contains a very helpful discussion of the coprozoic Protozoa of man, a sufficient knowledge of which is of utmost importance to those who actually examine stools for protozoan parasites; this subject has been only fragmentally treated in most books on parasitic Protozoa.

All drawings are magnified uniformly 2,000 diameters, which is enough for larger species but seems to be too small to show in detail the structures of more minute forms. The figures are very carefully and attractively done. The first plate shows five species of amoebae sketched beautifully in living and active condition and is worthy of special commendation.

NOTES

An event of great importance for parasitology was the opening last December of the Molteno Institute in Cambridge, England. The large and finely equipped building, made possible by the generosity of Mr. and Mrs. Percy A. Molteno, was dedicated exclusively to the promotion of Research in Parasitology. No more appropriate location could have been selected, for Professor G. H. F. Nuttall, despite inadequate support and under many other trying conditions, has built up in Cambridge a laboratory and center for parasitological study that has contributed researches of great practical and theoretical value and has given him an international reputation. Through his advice British and Colonial governments have profited greatly, both in war and in peace. In his establishment and maintenance of the widely known periodical *Parasitology,* originally a supplement to the Journal of Hygiene, the literature of this science has been greatly enriched and research in this field wisely and powerfully stimulated. All will agree that Dr. Nuttall has richly earned and will effectively utilize these new facilities. It is not feasible here to describe in detail the character of the new building which has ben carefully constructed with reference to its full adequacy for research. THE JOURNAL extends its congratulations to the University and to Dr. Nuttall on this splendid gift.

The Steel Memorial Medal for 1921 has been awarded to Dr. Albert Hassall, by the Council of the Royal College of Veterinary Surgeons. This medal is given every three years for scientific or literary work of merit in connection with the veterinary profession. Dr. Hassall has been in the Bureau of Animal Industry for thirty-five years, and in addition to publications on parasitology, has built up an Index Catalogue of Medical and Veterinary Zoology which is the most complete work of the sort in existence. The author and the subject catalogues were published as a joint work of Dr. Stiles and Dr. Hassall. These publications constitute very valuable reference works which are in great demand both in this country and abroad.

In a Monograph of the Existing Crinoids (U. S. Nat. Mus., Bull. 82, The Comatulids, Vol. 1, Pt. 1) A. H. Clark has extensive and interesting data on the parasites. Many organisms belonging to diverse groups are intimately associated with crinoids and the relations vary by imperceptible graduations from casual association to true parasitism. The author compares the relations of sessile marine organism and their parasites, with those of parasitic plants and their hosts. The parasites of crinoids are very highly specialized and the study of this monograph will repay those interested in the origin of the parasitic habit and its biological aspects.

The Université d'Alger, Algiers, Algeria, has established a new laboratory of parasitology. Dr. L. G. Seurat, formerly Professeur de Zoologic Générale, is in charge as Professuer de Zoologie Appliquée a la Factulté des Sciences.

The famous *Laws of Medicine* by Ibn Sina (Avicenna, 981-1037 A. D.) contains a section of great interest to helminthologists, viz., the last chapter on Diseases of the Intestine which deals with Entozoa. A recent admirable translation of this chapter (M. Khalil, *Jour. Trop. Med. and Hyg.*, Mar. 15, 1922, p. 65) shows that it furnishes a remarkably full and accurate diagnosis of four Entozoa, *Ascaris lumbricoides, Taenia saginata, Oxyuris vermicularis* and *Ancylostona duodenale.* The original author described with some care the symptoms and the remedies employed and in great part these hold good today, even though this be probably "the earliest valid record of the hookworm" yet noted. The commentary and biolographical notes added by the translator add greatly to the value of the article.